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(54) Title: LOCAL POLYMERIC GEL CELLULAR THERAPY (57) Abstract A method for providing a synthetic barrier made of biocompatible polymeric materials <i>in vivo</i> which involves application of a material to a tissue or cellular surface such as the interior surface of a blood vessel, tissue lumen or other hollow space, is disclosed herein. The material may also be applied to tissue contacting surfaces of implantable medical devices. The polymeric materials are characterized by a fluent state which allows application to and, preferably adhesion to, tissue lumen surfaces, which can be increased or altered to a second less fluent state <i>in situ</i> ; controlled permeability and degradability; and, in the preferred embodiments, incorporation of bioactive materials for release <i>in vivo</i> , either to the tissue lumen surface or to the interior of the lumen, which alter cell-to-cell interactions. It has also been discovered that tenascin is a mediator of smooth muscle cell migration through interaction with specific integrin components of the cells.		

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LOCAL POLYMERIC GEL CELLULAR THERAPY

Background of the Invention

This invention is generally in the area of methods of treating tissue defects and modulating cell to cell interactions by administration of a
5 polymeric gel material containing bioactive molecules to a tissue surface and the use of certain ECM and RGD peptides.

The hollow or tubular geometry of organs commonly has functional significance, for example,
10 in the facilitation of fluid or gas transport (blood, urine, lymph, oxygen or respiratory gasses) or cellular containment (ova, sperm). Disease processes may affect organ tissue or its components by encroaching upon, obstructing or otherwise
15 reducing the cross-sectional areas of the hollow or tubular elements. Additionally, other disease processes may violate the native boundaries of the hollow organ and thereby affect its barrier function and/or containment ability. These disease
20 processes include those which are induced by aging, diet, injury, or activation of the coagulation, complement and other inflammatory systems or the development of a neoplasia or malignancy. The ability of the organ or structure to properly
25 function can then be severely compromised. This is particular evident in coronary artery disease, where successful treatment initial may subsequently be complicated by overproliferation of endothelium, called restenosis, or vessel renarrowing or closing
30 after dilation.

A specific therapeutic strategy which would greatly benefit from an adjuvant treatment to prevent cell migration is percutaneous transluminal coronary angioplasty (PTCA, balloon angioplasty).
35 Balloon angioplasty has become the mainstay in the interventional therapy of advanced coronary and peripheral artery disease. While this procedure

achieves the therapeutic goal of enlargement of the diseased arterial lumen where a blockage occurs, the therapy can itself damage the arterial wall and cause alterations in arterial function. Restenosis or vessel reclosure following balloon angioplasty is the major limitation undermining a consistent long-term success rate for this procedure. In fact, the currently unmodifiable post-angioplasty failure rate due to restenosis is 30 to 50%.

Intimal hyperplasia or thickening of the vascular wall, a fundamental mechanism of restenosis, is caused by stimulation of smooth muscle cells within the wall, causing them to migrate, proliferate, and coordinately secrete or deposit extracellular matrix proteins. This combination of smooth muscle cell migration and matrix deposition, progressing toward the lumen, and eventually encroaching upon it, is responsible for restenosis. This SMC response to injury is marked by a transformation of SMC phenotype from a quiescent, contractile state to a synthetic, proliferative state in a high percentage of the medial SMCs. Another important event which occurs following injury is that SMCs (both synthetic and contractile SMCs) become migratory, moving from the media to the intima.

The types of problems associated with angioplasty are also characteristic of similar treatment of other types of natural lumens, including surgical correction and balloon dilation of urinary and reproductive tract disorders, for example, following prostate surgery, or treatment by laparoscopy and balloon dilation of stenosis or strictured fallopian tubes, as well as treatment of openings arising from disease, surgery and trauma. Further, these reobstructive problems also occur in artificially or therapeutically created lumens or

pathways, such as in renarrowing of the intrahepatic shunt formed in transjugular intrahepatic portosystemic shunting procedure (TIPS).

5 As described in the literature, for example, U.S. Patent No. 5,213,580 to Slepian, pre-formed polymeric materials can be inserted into blood vessels and then contoured to fit the surfaces of the vessels, providing protection of
10 the blood vessel and prevention of restenosis. As described in U.S. Patent Nos. 5,126,141 and 5,135,751 to Henry, et al., aqueous, thermally reversible gel compositions formed of a polyoxyalkylene polymer and an ionic polysaccharide
15 can be applied to injured areas of the body to prevent adhesions. These same type of polyoxyalkylene polymers have also been used for the local delivery of oligonucleotides (antisense) to the surgically exposed surface of blood vessels
20 for treatment of restenosis, as described by WO93/01286 by Rosenberg, et al.

None of these, however, describe a means
for forming a polymeric material at or on a lumen surface which can be used as a barrier of
25 controlled permeability or for controlled delivery of a bioactive substance, nor can these materials be targeted to a particular cell type. While the prior art discloses useful treatments of damaged lumen surfaces, it would be desirable to have
30 materials which could provide these additional useful functions, especially controlled permeability which would allow free exchange of gases and nutrients or controlled diffusion of macromolecules which are beneficial to the lumen
35 surface, as well as for controlled drug delivery to the surface, for example, of growth factors or antiinflammatories.

It is therefore an object of the present invention to provide polymeric materials which are initially amorphous, biocompatible, and can be formed *in situ*.

5 It is a further object of the present invention to provide polymeric materials of controlled permeability which can be used as selective barriers on lumen surfaces.

10 It is a still further object of the present invention to provide materials which can be used for controlled delivery of drugs and other biologically active substances, either to tissue lumen surfaces or into the lumens themselves.

Summary of the Invention

15 Methods for creating *in situ* specific local interactions or cellular interactions in living tissue are disclosed. This is accomplished by applying a fluent material which forms a local, selectively permeable barrier, alone or in
20 combination with specific bioactive molecules, directly to a site to be treated. Upon application, the fluent material is conformed to the tissue and converted to a less fluent state by alteration in temperature, ion concentration,
25 application of shear force, or chemical or physical polymerization or crosslinking. In one embodiment, cellular interactions, such as formation of thrombus, inflammation, or adhesions, are inhibited by physically blocking cellular and/or
30 macromolecular interactions while allowing selective permeability to nutrients, gases, and other molecules. Permeability is controlled by selection of the material, method of manufacture, density, degree of crosslinking, molecular weight
35 of monomer units, incorporation of particulate or other material, and degradability or non-

biodegradability of the polymeric material. In another embodiment, the polymeric gel is provided in combination with bioactive molecules, especially those providing contact guidance, or chemotactic or haptotactic activity, which can be utilized to alter cell proliferation, migration, and inflammatory reactions.

As demonstrated by the examples, a synthetic barrier made of a biocompatible polymeric material can be applied *in vivo* to a tissue or cellular surface such as the interior surface of a blood vessel or tissue lumen. The material may also be applied to tissue contacting surfaces of implantable medical devices. The polymeric material is applied in the first fluent state to the site to be treated using, for example, a catheter, or by means of spraying or irrigation at the time of surgery. The material is then reconfigured to have intimate conforming contact with the surface to be coated, and then maintained under conditions which convert the material into its second non-fluent state. The conversion may be achieved either by active methods in which the environment surrounding the material is altered by the addition or removal of chemicals or energy, or it may be by passive means in which, for example, maintaining the material at the normal internal body temperature of the patient causes the material to undergo conversion into its non-fluent state. The transition of the material from a fluent state to a non-fluent state may be the result of a phase change in which the material goes from a liquid state to a solid state, by gelation, or in the alternative, it may be the result of a viscosity change with the material actually remaining in a single phase.

As part of these studies, it has now been discovered that the extracellular matrix (ECM) protein tenascin facilitates cell migration *in vivo* for the treatment of diseased or injured tissues and can be used alone or in combination with a carrier such as the polymeric gel for localized therapy.

Brief Description of the Drawings

Figure 1 is a schematic of the method of the present invention.

Figure 2A is a cross sectional view of the multilumen features of the catheter shown in Figure 2B. Figures 2B and 2C are expanded views of catheters useful in the method described herein for application of polymeric materials to the tissue lumen surfaces.

Figures 3A-3G are schematics of photographs of application of a polymeric material as described herein within a mock hollow tubular organ. Figures 3A and 3B are schematics of the catheter and the catheter being inserted into the tube; Figure 3C is of the two balloons in the catheter being inflated to seal off the vessel; Figure 3D is of the polymeric material being injected into the tube; Figure 3E is of the tube with the polymeric material having gelled and the balloons deflated; Figure 3F is of the catheter being removed to leave a gel coating on the vessel walls with an interior lumen or annual space; and Figure 3G is of the lumen after the balloons are collapsed and withdrawn from the coated vessel, and the material has been smoothed and thinned by reapplication of the distal occlusion balloon.

Figures 4A-4F are schematics of micrographs of injection of polymeric material into isolated bovine coronary arteries.

Figure 5 is a schematic of a micrograph (250x) of the cross-section of a gel coated artery with a thin gel coating (lower left corner).

Figures 6A and 6B are scanning electron
5 micrographs of the intimal surface of rat carotid arteries following 60 minutes of reexposure to blood post-injury; Figure 6A is the control balloon abraded rat intimal (endoluminal) surface with significant platelet, white cell and fibrin
10 deposition; Figure 6B is the gel coated (Pluronic F127, 25% w/v) arterial surface showing a significant reduction in platelet, white cell and fibrin deposition and adherence.

Figure 7 is a schematic of photographs of the
15 effect of gel coating on limiting the development of arterial neointimal hyperplasia 14 days post-injury.

Figure 8 is a graph of % control migration versus peptide concentration (mM) for cyclic RGD
20 (closed squares), GRGDdSP (a stronger inhibitor of β_1 integrins) (open squares), GRGDSP (linear RGD peptide which inhibits β_1 integrins) (closed circles), and GRADSP (non-sense peptide) (open circles).

25 Figure 9A is a graph of the percent control migration of tenascin (1.0 $\mu\text{g/ml}$) versus untreated control.

Figure 9B is a graph of the percent control migration for 1 $\mu\text{g/ml}$ tenascin in combination with
30 either anti-vitronectin receptor or anti-fibronectin receptor, as compared with control tenascin alone.

Figure 10 is a graph of the SMC surface bound tenascin (1 mol/ 10^{-6} cells) versus soluble tenascin
35 in culture media ($\mu\text{g/ml}$).

Detailed Description of the Invention

As described herein, polymeric materials are applied to the surface of tissue lumens to provide a barrier having either a controlled permeability to materials in the lumen, for example blood, and/or controlled release of incorporated bioactive agents.

Selection of Polymeric Materials

The basic requirements for the polymeric material are biocompatibility and the capacity to be applied in a fluent state then chemically or physically reconfigured under conditions which can be achieved *in vivo* to yield a non-fluent polymeric material having defined characteristics in terms of permeability and release of incorporated materials.

The polymeric materials can be applied as monomers, macromers, polymers, or combinations thereof, maintained as solutions, suspensions, or dispersions, referred to herein jointly as "solutions" unless otherwise stated. Although capable of many forms in their non-fluent state, organogels and hydrogels represent preferred embodiments. Although non-degradable and biodegradable materials can be used, biodegradable materials are preferred. As used herein, "biodegradable" is intended to describe materials that are non-permanent and removed by natural or imposed therapeutic biological and/or chemical processes. For application to the interior of blood vessels following angioplasty, it is preferred to use polymers degrading substantially six months after implantation; for prevention of adhesions or controlled release following treatment for injury or surgery, the degradation should be correlated with the time required for healing, i.e., generally in excess of six days but less than six months.

The polymeric materials are selected from those materials which can be polymerized or their viscosity altered *in vivo* by application of exogenous means, for example, by application of
5 light, ultrasound, radiation, or chelation, alone or in the presence of added catalyst, or by endogenous means, for example, a change to physiological pH, diffusion of calcium ions (alginate) or borate ions (polyvinyl alcohol) into
10 the polymer, or change in temperature to body temperature (37°C).

As used herein, a hydrogel is defined as an aqueous phase with an interlaced polymeric component, with at least 60%, preferably at least
15 75%, more preferably with 80% or more, and as a specific example, with 90% of its weight as water. The following definition is from the Dictionary of Chemical Terms, 4th Ed., McGraw Hill (1989):
Hydrogel: a colloid in which the disperse phase
20 (colloid) has combined with the continuous phase (water) to produce a viscous jellylike product, for example, coagulated silicic acid.

An organogel is defined as an organic phase with an interlaced polymeric component, with at
25 least 60%, preferably at least 75%, more preferably with 80% or more, and as a specific example, with 90% of its weight as organic solvent. Preferred solvents include non-toxic organic solvents, including but not limited to dimethyl sulfoxide
30 (DMSO), and mineral and vegetable oils.

Suitable materials are commercially available or readily synthesizable using methods known to those skilled in the art. These materials include:

1. *Materials which polymerize or alter viscosity as a function of temperature or shear or other physical forces.*

Poly(oxyalkylene) polymers and copolymers
5 such as poly(ethylene oxide)-poly(propylene oxide) (PEO-PPO) or poly(ethylene oxide)-poly(butylene oxide) (PEO-PBO) copolymers, and copolymers and blends of these polymers with polymers such as poly(alpha-hydroxy acids), including but not
10 limited to lactic, glycolic and hydroxybutyric acids, polycaprolactones, and polyvalerolactones, can be synthesized or commercially obtained. For example, polyoxyalkylene copolymers are described by U.S. Patent Nos. 3,829,506; 3,535,307;
15 3,036,118; 2,979,578; 2,677,700; and 2,675,619, the teachings of which are incorporated herein.

Polyoxyalkylene copolymers are sold by BASF and others under the tradename PluronicTM. Preferred materials include F-127, F-108, and for
20 mixtures with other gel materials, F-68. These materials are applied as viscous solutions at room temperature or lower which solidify at the higher body temperature.

Other materials with this behavior are
25 known in the art, and can be utilized as described herein. These include KlucelTM (hydroxypropyl cellulose), and purified konjac glucomannan gum.

Polymer solutions that are liquid at an elevated temperature but solid or gelled at body
30 temperature can also be utilized. A variety of thermoreversible polymers are known, including natural gel-forming materials such as agarose, agar, furcellaran, beta-carrageenan, beta-1,3-glucans such as curdlan, gelatin, or
35 polyoxyalkylene containing compounds, as described above. Specific examples include thermosetting biodegradable polymers for *in vivo* use described in

U.S. Patent No. 4,938,763 to Dunn, et al., the teachings of which are incorporated herein.

Thixotropic and pseudoplastic polymers exhibit shear thinning, whereby the polymer becomes more fluent under shear, and then reverts to a high-viscosity or gelled form on cessation of shear. A preferred example of a material altering viscosity from a liquid to a gel upon exposure to shear or other physical forces is the naturally occurring hyaluronic acid, most preferably of a high molecular weight in the range of 300,000 daltons or more, at concentrations of about 1% or more. Hyaluronic is present in joints where it acts to absorb shock and lubricate the moving surfaces. This can also be crosslinked ionically, as discussed below.

2. *Materials which polymerize in the presence of ions or removal of ions.*

Tissue and blood contain numerous anions and cations, at regulated conditions of pH, ionic strength and osmolarity, which can induce the gelation or local precipitation of polymers. Several divalent ions including calcium, barium, magnesium, copper, and iron are normal constituents of the body tissues and blood. These ions can be used to ionically crosslink polymers, for example, alginates and derivatized alginates and kappa, lambda, and iota carrageenans will gel in the presence of calcium ions. Other carboxylated and sulfated polymers such as hyaluronic acid, heparin, carboxymethyl cellulose, cellulose sulfate, xanthan gum, and pectin and various natural gums such as traganth, can substantially increase in viscosity in the presence of divalent cations. Monovalent ions can gel gellan; potassium can gel kappa carrageenan. Chitosan is soluble in mildly acidic conditions, and will gel at physiological pH or with phosphate or sulfate ions. Organogels can

also be formed using these procedures. Typically the gelling polymer is dissolved in a tissue-compatible non-aqueous solvent and applied to tissue, where the polymers gels or precipitates as the organic solvent is removed by diffusion.

Materials which form polymers upon removal of ions, such as the salts of certain monomers or polymers, can also be used, where the salt diffuses or is diffused out of the monomer solution at the time of application to the tissue to be treated, or by addition of chelators such as ethylenediaminetetraacetic acid, EDTA, a chelating agent used to as an anticoagulant.

3. *Materials that can be crosslinked chemically or with light, ultrasound or radiation.*

Materials that can be crosslinked using light, ultrasound or radiation will generally be those materials which contain a double (ethylenic) bond or triple (acetylenic) bond, preferably with an electron withdrawing substituent attached to the double or triple bond, referred to inclusively herein as "unsaturated". Examples of suitable materials small molecules containing acrylic, allylic or vinyl groups, such as acrylic acid, vinyl acetate, vinyl pyrrolidone, allyl alcohol, and dimeric or oligomeric forms of these compounds such as methylene bisacrylamide. Preferred monomers are sufficiently large in molecular weight so as to not penetrate cells, typically 400 m.w., thereby minimizing toxicity. These include unsaturated derivatives of proteins, polysaccharides, or synthetic polymers. Biodegradable or non-biodegradable monomers may be used to form the polymer. Examples of suitable materials are described in WO93/17669 by Hubbell, et al., and U.S. Patent No. 4,938,763 to Dunn, et al., the teachings of which are incorporated

herein. Examples of preferred materials include the monomers which are polymerized into poly(acrylic acids) (i.e., CarbopolsTM), poly(acrylates), polyacrylamides, polyvinyl
5 alcohols, polyethylene glycols, and ethylene vinyl acetates.

Photopolymerization can be achieved directly by high energy photons provided, for example, ultraviolet, x-ray, or gamma radiation,
10 however agents which damage cells, typically ultraviolet less than 350 nm and gamma radiation, are to be avoided. Photopolymerization in visible light normally requires the presence of a photosensitizer, a substance that either increases
15 the rate of photoinitiated polymerization or shifts the wavelength at which polymerization occurs. Photoinitiation has advantages since can be used to limit the thickness of the gel formed by photopolymerization to a thin membrane.

20 4. *Materials that can be crosslinked by addition of covalent crosslinking agents such as glutaraldehyde.*

Any amino containing polymer can be covalently crosslinked using a dialdehyde such as
25 glutaraldehyde, or succindialdehyde, or carbodiimide (CDI). Examples of useful amino containing polymers include polypeptides and proteins such as albumin, elastin, and collagen, and polyethyleneimine. Peptides having specialized
30 function, as described below, can also be covalently bound to these materials, for example, using crosslinking agents, during polymerization.

Any of the foregoing materials can be mixed with other materials to improve their physiological
35 compatibility. These materials include buffers, physiological salts, conventional thickeners or viscosity modifying agents, fillers such as silica and cellulosics, and other known additives of

similar function, depending on the specific tissue to which the material is to be applied.

Determination of Permeability of Polymeric Materials

5 The polymeric material is designed to achieve a controlled permeability, either for control of materials within the lumen or for release of incorporated materials. There are basically three situations that the polymeric material is designed to achieve with respect to materials present in the lumen: wherein there is essentially passage of only nutrients (small molecular weight compounds) and gases from the lumen through the polymeric material to the tissue lumen surface or *vice versa*; wherein there is passage of nutrients, gases and selected macromolecules, including proteins and peptides; wherein there is passage of nutrients, gases, macromolecules and cells; and wherein the polymeric material serves as a barrier to passage. As used herein, "controlled porosity" refers to a defined porosity allowing passage only of certain intended molecules, or preventing passage of any molecules. The molecular weight ranges of these materials are known and can therefore be used to calculate the desired porosity. For example, a macromolecule can be defined as having a molecular weight of greater than 1000 daltons; cells generally range from 600-700 nm to 10 microns, with aggregates of 30-40 microns in size.

 This controlled permeability function of the polymeric material may be useful not only for direct transfer at one location but also for trapping or selective permeability downstream in a tissue lumen. For example, if an organ or tissue lumen secretes an endocrine factor upstream from the polymeric material, the polymeric material can serve as a selective trap to concentrate the

factor, to effect controlled release of the factor, or to prevent passage of the factor to the site covered by the polymeric material.

Solidification of polymeric material, by
5 gelation, viscosity change, phase change or polymerization, is generally referred to as "solidification" and yielding a "solidified material". Methods of achieving porosity control in the solidified material are known in the art.
10 An excellent review of controlled release systems and fabrication technology is provided in "Controlled Release Systems: Fabrication Technology" Vol. II, Dean Hsieh, Editor, Chapter 3 "Gels for Drug Delivery" by David W. Woodford and
15 Dean S.T. Hsieh pp. 42-57 (CRC Press, Florida), the teachings of which are incorporated herein.

Typically, porosity control is achieved by selection of the material to be solidified, i.e., chemical composition, molecular weight,
20 availability of groups for crosslinking; the degree of crosslinking of the polymer: ionic strength, osmolarity and pH of the polymer solution; addition of viscosity modifying agents such as sorbitol, glycerin or sucrose; addition of lipids or highly
25 charged polymers to alter surface binding to cells and proteins; and incorporation of water-insoluble organic material or particles. The latter can be used to form composites that have increased strength or form a gradient sieve.

30 Polymeric materials can also be applied in layers of different or gradient porosity, or encapsulating bioactive materials, in the same or staggered layers for cyclic release. Release of incorporated biologically active materials is
35 described below in more detail.

Incorporation of Bioactive Agents

1. Selection of Bioactive Agents

A wide variety of bioactive agents can be incorporated into the polymeric material. These can be physically or chemically incorporated into the polymeric material. Release of the physically incorporated material is achieved by diffusion and/or degradation of the polymeric material; release of the chemically incorporated material is achieved by degradation of the polymer or of a chemical link coupling the agent to the polymer, for example, a peptide which is cleaved *in vivo* by an enzyme such as trypsin, thrombin or collagenase. In some cases, it may be desirable for the bioactive agent to remain associated with the polymeric material permanently or for an extended period, until after the polymeric material has degraded and removed from the site.

In the broadest sense, the bioactive materials can include proteins (as defined herein, including peptides unless otherwise specified), saccharides, polysaccharides and carbohydrates, nucleic acids, lipids, gangliosides, and synthetic organic and inorganic materials.

Specific materials include antibiotics, antivirals, antiinflammatories, both steroidal and non-steroidal, antineoplastics, anti-spasmodics including channel blockers, modulators of cell-extracellular matrix interactions including cell growth inhibitors and anti-adhesion molecules, enzymes and enzyme inhibitors, anticoagulants and/or antithrombotic agents, growth factors, DNA, RNA, inhibitors of DNA, RNA or protein synthesis, compounds modulating cell migration, proliferation and/or growth, vasodilating agents, and other drugs commonly used for the treatment of injury to tissue. Specific examples of these compounds include angiotensin converting enzyme inhibitors, prostacyclin, heparin, salicylates, nitrates,

calcium channel blocking drugs, streptokinase, urokinase, tissue plasminogen activator (TPA) and anisoylated plasminogen activator (TPA) and anisoylated plasminogen-streptokinase activator complex (APSAC), colchicine and alkylating agents, and aptomers. Specific examples of modulators of cell interactions include interleukins, platelet derived growth factor, acidic and basic fibroblast growth factor (FGF), transformation growth factor β (TGF β), epidermal growth factor (EGF), insulin-like growth factor, and antibodies thereto. Specific examples of nucleic acids include antisense and ribozymes. Specific examples of other bioactive agents include modified extracellular matrix components or their receptors, and lipid and cholesterol sequestrants.

In a preferred embodiment, the bioactive materials are selected to provide chemotactic activity, haptotactic activity, or contact guidance for cells. Chemotaxis is defined as directed migration in response to a concentration gradient of a soluble attractant, i.e., in the gel. A definition is provided in "The Molecular and Cellular Biology of Wound Repair" ed. R.A.F. Clark and P.M. Henson ed., (Plenum Press, NY 1988) Chapter 13. J.B. McCarthy, Sas, and Furcht, the teachings of which are incorporated in. Haptotaxis is defined as the directed migration along an adhesion gradient. Information comes from the substratum; as described herein, by incorporation into the polymeric material of molecules that direct the behavior of the cells. Examples include extracellular matrix proteins such as laminin, fibronectin, vitronectin or collagen, or peptides derived therefrom or having an effect on binding to the proteins, such as the RGD peptides described in the following examples. Contact guidance refers

to the physical direction of cells, through grooves, fissures, or pores of the polymeric material, or by incorporation within the polymeric material of particles, ribbons, or fibers which
5 direct cell growth. An example is regeneration of nerve fibers, which does not occur in the absence of physical guidance, as in the form of a sheath.

In applications where multiple polymer layers are used, different pharmacological agents
10 can be employed in different polymer layers to achieve specific effects.

Optional additions to the polymeric material such as barium, iodine or tantalum salts for X-ray radio-opacity allow visualization and
15 monitoring of the coating.

Cells can also be incorporated into the polymeric solution as a suspension which forms a gel at the tissue surface that allows the cells to grow and in some cases to proliferate. The cells
20 can be living (whether naturally occurring or produced through recombinant DNA technology), artificial cells, cell ghosts (i.e., RBC- or platelet ghosts), or pseudovirions, to serve any of several purposes. For example, the cells may be
25 selected to produce specific agents such as growth factors at the local tissue location.

Cells incorporated in the material may also be progenitor cells corresponding to the type of tissue at the treatment location or other cells
30 providing therapeutic advantages. For example, liver cells might be incorporated into the polymeric material and implanted in a lumen created in the liver of a patient to facilitate regeneration and closure of that lumen. This might
35 be an appropriate therapy in cases where diseases (e.g. cirrhosis, fibrosis, cystic disease or malignancy) results in non-functional tissue, scar

formation or tissue replacement with cancerous cells. Similar methods may be applied to other organs as well.

5 2. *Physical Incorporation of Bioactive Agents.*

In most cases, it is possible to physically incorporate the bioactive agent by mixing with the material prior to application to the tissue surface and polymerization. The material can be mixed into
10 the monomer solution to form a solution, suspension or dispersion. In one embodiment, the bioactive agent can be encapsulated within delivery devices such as microspheres, microcapsules, liposomes, cell ghosts or pseudovirions, which in themselves
15 effect release rates and uptake by cells such as phagocytic cells.

 3. *Chemical Incorporation of Bioactive Agents.*

Bioactive agents can be chemically coupled
20 to the polymeric material, before or at the time of polymerization. In the preferred embodiment, the bioactive agents are chemically coupled prior to administration of the polymeric material to the tissue surface. Several polymeric biocompatible
25 materials are amenable to surface modification in which surface bound bioactive molecules/ligands exhibit cellular binding properties. These methods are described by Tay, Merrill, Salzman and Lindon in Biomaterials 10, 11-15 (1989), the teachings of
30 which are incorporated herein by reference.

Covalent linkages can be formed by reacting the anhydride or acid halide form of an N-protected amino acid, poly(amino acid) (two to ten amino acids), peptide (greater than 10 to 100 amino
35 acids), or protein with a hydroxyl, thiol, or amine group on a polymer. The amine groups on the amino acid or peptide must be protected before forming the acid halide or anhydride, to prevent self-

condensation. N-protection is well known by those skilled in the art, and can be accomplished by use of various protecting groups, such as a carbobenzoxy (CBZ) group.

5 The term "protecting group" as used herein refers to a moiety which blocks a functional group from reaction, and which is cleavable when there is no longer a need to protect the functional group. Examples of functional groups include, but are not
10 limited to, amino, hydroxy, thio, and carboxylate groups. Examples of protecting groups are well known to those skilled in the art.

 A carboxylate-containing compound can contain various functional groups, such as hydroxy,
15 thio, and amino groups, that can react with an acid halide or anhydride. These functional groups must be protected before forming an acid chloride or anhydride to avoid self-condensation. After
20 formation of the acid chloride or anhydride, and subsequent reaction with the hydroxyl, thiol, or amino group(s) on another molecule, the protecting group can be removed in a "deprotecting" step.—The
N-protected amino groups can be deprotected by means known to those skilled in the art. Any
25 hydroxy or thio groups on these compounds must be protected so as not to react with the acid halides or anhydrides. Examples of suitable protecting groups for alcohols include but are not limited to trialkyl silyl groups, benzyl ethers, and
30 tetrahydropyranyl ethers. These groups can be protected by means known to those skilled in the art, and can be subsequently deprotected after the esterification is complete. Examples of protecting groups can be found in Greene, T.W., and Wuts.,
35 P.G.M., "Protective Groups in Organic Synthesis, 2d Ed., John Wiley & Sons, Inc., pp. 317-318 (1991), hereby incorporated by reference.

A non-limiting method for preparation of acid halide derivatives is to react the carboxylic acid with thionyl chloride, preferably in benzene or toluene with a catalytic amount of DMF. A known
5 method for producing anhydrides is to react the carboxylic acid with acetic anhydride. In this reaction, as acetic acid is formed, it is distilled out of the reaction vessel. Peptides can be covalently bound to the polymeric material, for
10 example, when the polymeric material is a polymer of an alpha hydroxy acid such as poly(lactic acid), by protecting the amine functionality on the peptide, forming an acid halide or anhydride of the acid portion of the polymer, reacting the acid
15 halide or anhydride with free hydroxy, thiol, or amine groups on the polymer, then deprotecting the amine groups on the peptide to yield polymer having peptide bound thereto via esterification, thioesterification, or amidation. The peptide can
20 also be bound to the polymer via a free amine using reductive amination with a dialdehyde such as glutaraldehyde.

The ester groups on a polyester surface can be hydrolyzed to give active hydroxy and carboxyl
25 groups. These groups can be used to couple bioactive molecules. Preferably, before converting the active carboxylate group to the acid halide or anhydride form, the active hydroxy group is protected to avoid reaction with the resulting acid
30 halide or anhydride. As a non-limiting example, the active hydroxy group can be protected as a benzyl ether. The active carboxyl group can then be converted to the acid halide or anhydride, and reacted with a hydroxy or amino group on a second
35 compound to form an ester or amide linkage. The O-protected hydroxy group can then be deprotected.

Polyanhydrides can be partially hydrolyzed to provide carboxyl groups. The resulting carboxyl groups can be converted to acid halides, which can be reacted with amino acids, peptides, or other amine containing compounds with binding properties and form an amide linkage.

Polyesters and polylactones can be partially hydrolyzed to free hydroxyl and carboxyl groups. The hydroxyl groups can be protected by means known to those skilled in the art, and the carboxyl groups converted to acid halides. The acid halides can be reacted with amino acids, peptides, or other amine containing compounds with binding properties and form an amide linkage.

Alternatively, if the hydroxyl groups are primary or secondary hydroxyl groups, they can be oxidized to aldehydes or ketones, and reacted with amines via reductive amination to form a covalent linkage.

Polyamides can be partially hydrolyzed to provide free amine and carboxylic acid groups. The amine group can then be reacted with an amino acid or peptide in which the amine groups have been protected, and the carboxyl groups have been converted to acid halides. Alternatively, the amine groups on the polyamide can be protected, and the carboxyl groups converted to acid halides. The resulting acid halides can then be reacted directly with the amine groups on amino acids or peptides.

Polyalcohols with terminal hydroxy groups can be appended with amino acids or peptides. One first protects the amine groups, then converts the carboxyl groups on the amino acid or peptide to acid halides. The acid halide can be reacted directly with the hydroxy group to provide an ester linkage.

The acid halides described above can also be reacted with thiol groups to form thioesters.

Application of the Polymeric Materials

5 1. *Administration of polymeric material to tissue surfaces.*

In general terms, the polymeric material is a biocompatible polymeric material having a variable degree of fluency in response to a stimulus, as described above. The material is such
10 that it is substantially non-fluent *in vivo* upon completion of the coating process. The material, in its fluent form, is positioned in contact with a tissue or cellular surface to be coated and then stimulated to render it non-fluent, as described
15 above. The fluent phase of the polymeric material is applied using catheters, endoscopes, syringes, or sprays, depending on the tissue lumen surface to which it is applied. Such devices are known to those skilled in the art.

20 The coating typically will be applied using some type of catheter, such as a modified PTCA catheter. The material is preferably applied using
a single catheter with single or multiple balloons and lumens. The catheter should be of relatively
25 low cross-sectional area. A long thin tubular catheter manipulated using fluoroscopic guidance is preferred for providing access to the interior of organ or vascular areas.

30 The tissues involved may be those organs or structures having hollow or tubular geometry, in which case the polymeric products are deposited within the naturally occurring lumen.

Alternatively, the tissue may be a normally solid organ in which a cavity has been created either as
35 a result of a surgical procedure, a percutaneous intervention, an accidental trauma, or disease. Examples of hollow vessels include the aorta, coronary arteries, veins and lymphatic vessels.

Examples of hollow organs include the heart, the eye, intestine, fallopian tube, uterus, kidney or the bladder. In addition many organs have component structures which are hollow such as the trachea (lung), the biliary duct (gall bladder), or the pancreatic duct (pancreas). In addition to organs around hollow geometrics many solid organs possess internal "true" spaces, such as cavities, cavernous sinuses or lumens, or "potential" spaces, following a disease process which creates the space, i.e., the interior of a necrotic tumor.

Once the fluid phase of the polymeric material has been applied, the fluid state of the material is reconfigured to form a coating or "paving" layer in intimate and conforming contact with the surface. The resulting paving layer can have a sealing function, i.e., it forms a coating of sufficiently low porosity that it excludes macromolecules (i.e., less than 53 Angstroms for a small protein up to 2000 Angstroms for a rod such as myosin) and cells (600 nm for platelets up to 30 to 40 microns for large cells). The coating preferably has a thickness on the tissue surface on the order of 0.001-1.0 mm, however, coatings having a thickness outside this range may be used as well. By appropriate selection of the material employed, using materials commercially available, and methods for crosslinking that are known to yield a specific percent crosslinking and porosity, and of the configuration of the paving material, the process can be tailored to satisfy a wide variety of biological or clinical situations.

The polymeric materials may be applied in custom designs, with varying thicknesses, lengths, and three-dimensional geometries (e.g. spot, stellate, linear, cylindrical, arcuate, spiral) to achieve varying finished geometries. Further, the

process may be used to apply material to the inner surfaces of hollow, cavernous, or tubular biological structures (whether natural or artificially formed) in either single or multi-layer configurations. The process may also be used, where appropriate, to occlude a tissue lumen completely.

2. *Application of Polymeric Material to Isolated Cells and cell aggregates.*

The polymeric material may also be applied to cellular surfaces, for example to coat or encapsulate individual or multiple cells such as blood components, smooth muscle cells, endothelial cells and tumor cells that are being removed and are treated to prevent attachment if accidentally detached and left in the patient. In general, this methodology would be used to isolate the treated cells.

In a second embodiment, the polymeric material is used to protect and attach isolated cells or cell aggregates to an area within the body where cell attachment, growth and/or proliferation is desirable. One process involves first inserting a catheter into a lumen within a diseased organ segment. The lumen can be a native vessel or it can be a man-made lumen. A polymeric plug is introduced into the lumen. The catheter is then removed, leaving the plug in place to act as a focus for new growth stemming from cells implanted along with the polymeric plug. If the desire is for a more tubular structure, the plug can be appropriately reconfigured.

3. *Representative Devices for application of polymeric material.*

Figure 1 is a schematic of the process for applying polymeric material to a tissue lumen. In step 1, a lesion in the lumen is identified and isolated. In step 2, a catheter, optionally a

balloon catheter consisting of a tubular shaft which includes one or more balloons, is inserted into the lumen. In the preferred embodiment for the treatment of blood vessels, the distal occlusion balloon is used to occlude the distal end of a treatment site. In embodiments where the lumen can be rinsed clean, for example at an end artery or in the gastrointestinal tract or lungs, it is not necessary to inflate the balloon. In any case, the treatment site is cleared of blood, mucous, or other extraneous material, as shown in step 3. The site may then be treated with drugs, for example a drug inhibiting responsiveness to mechanical stimuli or cell proliferation, as shown in step 4. In step 5, if appropriate, the lesion itself is treated by expansion of the balloon, in the case of an arterial plaque, or by other mechanical, thermal, optical, photochemical, ultrasonic, or radiation means. As shown in step 6, the site is again treated with drugs and/or washed or compounds to increase adhesiveness applied. In step 7, the solution for forming the polymeric material at the tissue surface is applied and polymerized or solidified. In some embodiments the catheter includes a "mold core" which is used to shape the polymeric material so that it covers only the area to be treated in a thin layer. The central mold core member may be able to adjust size, i.e., for a balloon it may be underinflated to not occupy the maximum space, thereby leaving room for the polymeric material. The polymeric material may be shaped as a uniform layer, or patterned or segmented as desired. In step 8, the catheter is removed and flow of material through the polymeric coated lumen restored.

Two other embodiments of delivery catheters that can be utilized for application of the

polymeric material are shown in Figures 2A, 2B and 2C. Figure 2A is a single entity with means for entering a tissue lumen, isolating a zone, washing, applying a drug, adhesive and/or a polymeric material and a core forming member and/or dilating member. The catheter 11 is constructed with two isolation balloons 10, 14, and a central dilating or molding balloon 12, as well as a plurality of lumens and an attached reservoir 16 for delivering washing fluid, drug, adhesive and/or polymer. A detailed cross-section enlargement of the tip of the application device is shown in Figure 2A. Two isolation balloons 18, 22 are constructed out of elastomeric material, i.e., latex, krayton or C-flex or thermoplastic polymers such as polyethylene, polyolefin co-polymer, polyethylene terephthalate, or nylon. The balloons 18, 22 are attached to a multi-lumen shaft 43 including a central lumen 42 running the length of the device to allow flushing or passage over a guide wire (not shown). A central mold-core balloon 20 is fabricated out of similar materials to those forming the isolation balloons 18, 22, or from less compliant materials so that it opens to a designated dimensions without a continuous stretch or expansion via creep of the balloon material. In addition, lumens exist for filling the isolation balloon 24, 26 and for instilling, filling or removing fluid from the dilating or mold core balloons 32, 34. In addition, there are lumens 30, 36 for instilling fluid into the isolation zone. Lumens 38, 40 are used to instill fluid or remove fluid from the isolation zone. This device provides a means to instill, perfuse, or superfuse a zone.

Figure 2C shows another catheter 45 encompassing two telescoping members 46 within 44.

Zone isolation balloons 50 and 52 and a central mold core and/or dilating balloon 54, as well as instillation or aspiration ports 56, provide an alternative means for applying polymeric material.

5 The material may also be applied to the surface to be coated by spraying, extruding or otherwise internally delivering the material in a fluent form via a delivery device having single or multiple lumens.

10 Application of the coating material may be accomplished by extruding a solution, dispersion, or suspension of monomers, polymers, macromers, or combinations thereof through a catheter to coat or fill a tissue or cellular surface, a tissue lumen
15 or a hollow space. The formation of the coating can be controlled by introducing crosslinking agents, gelling agents or crosslinking catalysts together with the fluent material and then altering the conditions such that crosslinking and/or
20 gelling occurs. Thus, when a balloon catheter is used, a flow of heated or chilled fluid into the balloon can alter the local temperature to a level
at which gelling or cross-linking of introduced material is induced, thereby rendering the material
25 non-fluent. Localized heating or cooling can be enhanced by providing a flow of heated or chilled liquid directly onto the treatment site. Thermal control can also be provided, however, using a fluid flow through or into the balloon, or using a
30 partially perforated balloon such that temperature control fluid passes through the balloon into the lumen. Thermal control can also be provided using electrical resistance heating via a wire running along the length of the catheter body in contact
35 with resistive heating elements. This type of heating element can make use of DC or radio frequency (RF) current or external RF or microwave

radiation. Other methods of achieving temperature control can also be used, including light-induced heating using an internal optical fiber (naked or lensed). Similar devices can be used for
5 application of light, ultrasound, or irradiation.

Catheter bodies are made of standard materials, including metals such as surgical steel and thermoplastic polymers. Occluding balloons may be made from compliant materials such as latex or
10 silicone, or non-compliant materials such as polyethylene terephthalate (PET). The expansible member is preferably made from non-compliant materials such as PET, (PVC), polyethylene or nylon. If used, the balloon catheter portion of a
15 dilatation may optionally be coated with materials such as silicones, polytetrafluoroethylene (PTFE), hydrophilic materials like hydrated hydrogels and other lubricous materials to aid in separation of the polymer coating.

20 Medical Indications for Treatment

1. *Treatment of Lumen Surfaces*

In addition to treatment of arteries, the method described herein can be utilized for other applications such as paving the interior of veins,
25 ureters, urethras, bronchi, biliary and pancreatic duct systems, the gut, nasolacrimal ducts, sinus cavities, the eye, and eustachian, spermatoc and fallopian tubes. The process can be used to provide a paving layer in the context of
30 transjugular intrahepatic portosystemic shunting procedure (TIPS), dialysis grafts, arterio-venous fistulae, and aortic and other arterial aneurysms, as well as in the treatment of abrupt vessel reclosure post PCTA, the "patching" of significant
35 vessel dissection, the sealing of vessel wall "flaps" either secondary to catheter injury or

spontaneously occurring, and the sealing of aneurysmal coronary dilations associated with various arteritidies.

5 The ultimate *in vivo* geometry of the material dictates the final function of the coating. The thinner applications allow the polymer film to function as a coating, sealant, partitioning barrier, bandage, and/or drug depot.

10 The hollow or cavernous geometry present in many body components has functional significance. Such geometry facilitates fluid (blood, urine, lymph, bile) gas, a cellular (ovary, spleen) containment or transport. These hollow vessels, organs and organ components are typically composed of several tissue layers. Generically these organs are composed of an inner cellular layer typically functioning as a barrier layer, one or several middle functional layers containing muscularis, glands or other functional tissue, and an external
15 supportive or stromal covering layer.
20

 Disease may effect the innermost layer of these hollow organs and thereby violate their barrier function. Diseases can be either: (1) systemic with overall diffuse constitutional
25 manifestations, (2) systemic with localized specific intra-organ focal involvement, or (3) localized only with definitive regional intra-organ involvement. Examples of such diseases include spontaneous plaque rupture, unstable angina, non-cardiogenic pulmonary edema, sepsis, and
30 erosive/infiltrative tumors.

2. *Manipulation of Cell-Cell Interactions*

 The methods described herein restore the barrier function, and/or provided controlled drug
35 delivery, thereby providing a method for treatment for these disorders. The polymeric material can also served as a trophic layer, an adhesive layer,

as a coating of other therapeutic intraluminal devices, as an absorbing layer, as a sequestrant, or chelator.

As described above, in a particularly preferred embodiment, the polymeric material is used to apply an effective amount of bioactive molecules such as chemotactic molecules, haptotactic molecules or molecules providing contact guidance, to a site where the bioactive molecules would otherwise not reach in an effective dosage. In the case of cell to cell interactions, the polymeric materials provide a substrate that is analogous to the cell surfaces on which these molecules are normally found and therefore appear to be significantly more effective than administered in the same dosage in the absence of the polymeric material.

Materials such as attachment peptides, selectin receptors and carbohydrate molecules such as Sialyl Le^x, can be used which serve to attract and bind specific cell types, such as white cells and platelets. Materials such as fibronectin, vitronectin, and collagen, can be used to non-specifically bind cell types, to facilitate cell migration and thereby to enhance healing. Growth factors and modulators of cell growth, proliferation and migration are particularly useful. For example, one may incorporate into the polymeric material a chemoattractant factor to cells such as PDGF or matrix proteins, i.e., fibronectin, laminin, fibrin, or type IV collagen, which will then facilitate cell ingrowth for wound repair or a gap or rent resulting from disease.

Extracellular Matrix Components

During the past two decades, the base knowledge of cell adhesion and migration in extracellular matrices (ECMs) at the molecular

level has expanded rapidly. Early efforts in this area of research concentrated on the adhesion-promoting ECM protein fibronectin (FN). Studies which employed limited proteolysis of FN revealed a
5 120 KD polypeptide fragment of FN which supported cell adhesion in a way similar to the whole molecule. This fragment existed as a domain embedded in the FN molecule and was designated the cell-binding domain. Further sequence analyses and
10 peptide mapping of the FN cell-binding domain yielded a minimal sequence which maintained cell-binding activity in the tetrapeptide Arg-Gly-Asp-Ser (RGDS).

The biological interaction of the RGDS
15 sequence with cell-surface fibronectin receptors was revealed by demonstrating that synthetic RGDS-containing peptides in solution could competitively inhibit fibroblast cell spreading on fibronectin-coated substrates. Soluble RGDS also inhibited the
20 direct binding of radiolabeled fibronectin to fibroblastic cells in suspension. These competition studies indicated that the RGD sequence is critical for the cell adhesive function of the parent molecule.

25 After the RGD cell adhesion recognition site in fibronectin was identified, the sequences of other cell adhesion proteins were examined for related signals. Other proteins known to carry functional RGD sequences include the platelet
30 adhesion proteins fibrinogen, vitronectin and von Willebrand factor, osteopontin, and laminin. These findings imply that RGD is a ubiquitous cell adhesion signal.

Specific RGD peptides are described in U.S.
35 Patent Nos. 4,517,686 to Ruoslahti, et al., 4,589,881 to Pierschbacher, et al., 5,169,930 to Ruoslahti, et al., 5,149,780 to Plow, et al.,

4,578,079 to Ruoslahti, et al., 5,041,380 to Ruoslahti, et al., and Pierschbacher and Ruoslahti, J. Biol. Chem. 262(36), 17294-17298 (1987), Mohri, et al., Amer. J. Hem. 37:14-19 (1991), Aumailley, et al., FEBS 291(1), 50-54 (1991), Gurrath, et al., Eur. J. Biochem. 210, 911-921 (1992), and Scarborough, et al., J. Biol. Chem. 268(2), 1066-1073 (1993), the teachings of which are incorporated herein.

10 Laminin is a large adhesive glycoprotein found in basement membranes which promotes cell adhesion, migration, differentiation, and growth (Kleinman, et al., J. Cell Biochem. 27:317-325 (1985); Kleinman, et al., Biochem. 25:312-318 (1986); Beck, et al., FASEB J. 4:148-160 (1990).
15 LN is composed of three chains designated A (M_r = 400 kD), B1 (M_r = 210 kD), and B2 (M_r = 200 kD). All three chains of the murine protein have been cloned and sequenced (Sasaki & Yamada, J. Biol.
20 Chem. 262:17111-17117 (1987); Sasaki, et al., Proc. Natl. Acad. Sci. USA 84:935-939 (1987); Sasaki, et al., J. Biol. Chem. 263:16536-16544 (1988), and several adhesion-promoting sites were identified on the molecule. Several synthetic peptides based on
25 sequences have been described as having biological activities similar to those of the whole laminin molecule. A nonapeptide CDPYIGSR as well as the pentapeptide YIGSR, from the B1 chain were shown to promote cell attachment and migration (Graf, et
30 al., Cell 48:989-996 (1987), Biochem. 26:6896-6900 (1987)). Further studies have shown that YIGSR-containing peptides can inhibit angiogenesis and tumor metastasis (Grant, et al., Cell 58:933-943 (1989), Iwamoto, et al., Science 238:1132-1134 (1987), Sakamoto, et al., Cancer Res. 51:903-906
35 (1991). Other peptides include PDSGR and IKVAV.

The YIGSR peptide class of adhesion ligands is a good example of a class of compounds which can be utilized for the treatment of diseases where cell proliferation and migration in the affected tissues occurs. While YIGSR peptides have been shown to selectively inhibit specific cell-ECM interactions, they must reach their preselected and specific target tissues in order to be therapeutically effective. Systematic administration of YIGSR would typically be an unsatisfactory therapeutic strategy since significant interference with normal cell-ECM interactions as well as those of targeted cells would occur. A more appropriate therapy would be to deliver YIGSR locally to the targeted site.

Integrin receptors for ECM

Isolation of RGD-directed cell-surface receptors for various cell adhesion proteins from many cell types has been performed using affinity chromatography on SepharoseTM carrying the appropriate, covalently bound, adhesion protein. Cell-surface adhesion receptors from cell extracts were observed to specifically bind to these columns and were eluted with RGD-containing peptide solutions. The use of fibronectin as the affinity ligand yielded a receptor that was a heterodimer with a 160 kD α -subunit and a 140 kD β -subunit. Similar affinity chromatography experiments have yielded distinct heterodimeric RGD-directed receptors specific for vitronectin and a platelet receptor with affinities for fibrinogen and fibronectin. It was realized that the heterodimeric structure was characteristic of RGD-directed receptors, with α -subunits ranging between 140 and 160 kD and β -subunits ranging between 90 and 140 kD. These RGD receptors, known as

integrins, form the integrin superfamily of cell-surface adhesion proteins.

The integrin superfamily is an important and well characterized group of cell-surface
5 receptors for both cell-substrate and cell-cell adhesion. Integrins are characteristically membrane-spanning heterodimeric protein complexes consisting of an α -subunit and a β -subunit. Fourteen distinct α -subunits and 11 β -subunits have
10 currently been isolated and identified, and several $\alpha\beta$ combinations have been observed. Integrin complexes containing β_1 and β_3 subunits generally are involved in cell adhesion to the extracellular matrix, while the β_2 integrins are involved in cell-
15 cell adhesion.

Integrins typically bind to cell adhesion proteins via the rather highly conserved sequence Arg-Gly-Asp X (RGDX), where X is variant depending on the particular cell adhesion protein. It was
20 observed that by varying this flanking residue, the affinity of the RGDX ligand for particular integrins was modified, but selectivity for specific integrins was not achieved. Further studies indicated that cyclization of RGDX-
25 containing peptides created a ligand which was highly selective for integrin $\alpha\nu\beta_3$, the vitronectin receptor. Other studies confirmed that RGD sequences that are conformationally constrained within cyclic peptides bound with higher affinity
30 and selectivity for integrin $\alpha\nu\beta_3$ than linear RGD sequences. Extracellular administration of cyclic RGD peptides has been shown to inhibit cell adhesion and migration on vitronectin-coated substrates *in vitro*.

35 A recent *in vitro* study examined the role of β_1 and $\nu\beta_3$ integrin receptors in promoting SMC adhesion and migration on substrates coated with

fibronectin (FN), laminin (LN), vitronectin (VN), type I collagen (I), and type IV collagen (IV). Using functionally blocking antibodies directed against specific integrin complexes, Clyman et al.,
5 Exp. Cell Res. 200:272-284 (1992), found that SMC adhesion on the FN-, LN-, VN-, I-, or IV-coated substrates depended exclusively on functioning β_1 integrins and that SMC migration on these substrates depended to a large extent on the $\alpha\nu\beta_3$
10 integrin.

Ligand affinity chromatography and immunoprecipitation analyses identified a unique series of β_1 integrins binding to each matrix component: FN ($\alpha_5\beta_1$, $\alpha_3\beta_1$, $\alpha\nu\beta_1$), LN ($\alpha_1\beta_1$, $\alpha_7\beta_1$),
15 VN($\alpha\nu\beta_1$), I ($\alpha_1\beta_1$, $\alpha_2\beta_1$), and IV ($\alpha_1\beta_1$). The β_3 integrin, $\alpha\nu\beta_3$, was observed to bind to all of the adhesion proteins tested (FN, LN, VN, I, and IV). These studies suggested that induction of SMC migration required a switch from an immobile state,
20 consisting of stable β_1 integrin interactions with the ECM, to a mobile state, where cells form transient interactions with the ECM via integrin $\alpha\nu\beta_3$, and that cyclic RGD should be a potent inhibitor of SMC migration since it could
25 specifically block integrin $\alpha\nu\beta_3$ interactions with the ECM. This has now been demonstrated, as shown by the following examples.

Tenascin

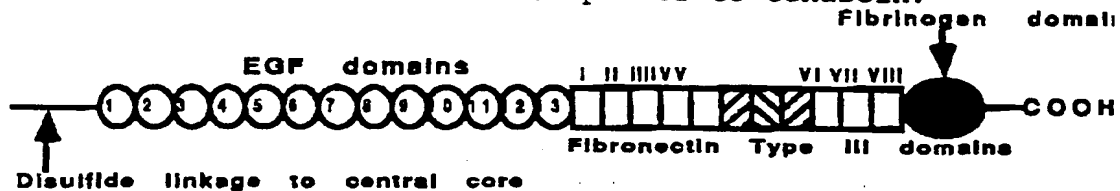
Tenascin is an unusually large hexameric
30 ECM protein of molecular weight greater than 1000 kDa, when compared to other ECM proteins such as fibronectin which is dimeric and 400 kDa. Electron microscopy of tenascin molecules reveals a characteristic six-armed structure with a central
35 globular domain, as reported by Chiquet-Ehrismann, FASEB J. 4:2598-2604 (1990). The distribution of tenascin in tissues is much more restricted than

that of laminin and fibronectin. Recent evidence has revealed that tenascin is transiently expressed in many developing organs during organogenesis, in the stroma of specific tumors, and in adult tissues during wound healing, as reported by Mackie, et al., J. Cell Biol. 107:2757-2767 (1988), Chuong and Chen, Am. J. Path. 138:427-440 (1991). These findings have led to investigations of the functional role of tenascin during these processes.

The earliest studies of tenascin function primarily focused on functional domains of tenascin and their effects on cell-matrix adhesion or interactions of tenascin with ECM components, as described by Chiquet-Ehrismann (1990; 1991). A major cell binding functional domain of tenascin was mapped with a monoclonal antibody, mAb Tn68, by Chiquet-Ehrisman, et al., Cell 53:383-390 (1988). The Tn68 epitope peptide has been demonstrated by Prieto, et al., J. Cell Biol. 119:663-678 (1992), to promote fibroblast adhesion when it is adsorbed to culture substrates. In contrast to the adhesion-promoting activity of the Tn68 epitope peptide, the whole tenascin molecule inhibits fibronectin- or tenascin-mediated cell adhesion *in vitro*. Further studies by Prieto, et al., have shown that the monoclonal antibody directed against the Tn68 epitope, mAb Tn68, blocks the inhibition of tenascin-mediated cell adhesion on fibronectin substrates. These studies indicate that the TN68 epitope is anti-adhesive in its native state within the whole tenascin molecule but can promote cell adhesion as a peptide fragment.

More recent functional mapping studies of tenascin revealed four independent cell binding domains along the arm of a tenascin molecule, as diagrammed below. The fibrinogen-like domain at the C-terminal knob of the arm and a domain

containing fibronectin type III repeats II-VI promote cell adhesion, as described by Prieto, et al. The EGF-like repeats and the last two fibronectin type III repeats were observed to inhibit cell adhesion. Other studies reported by Aukhil, et al., *J. Biol. Chem.* 268:2542-2553 (1993), revealed that a domain containing fibronectin type III repeats IV-V and the fibrinogen domain had heparin-binding as well as cell-binding activities. These studies provide a basis for the multifunctional role of tenascin in the ECM and guidelines for isolating receptors that mediate various cellular responses to tenascin.



Disulfide linkage to central core

*Adapted from Aukhil et al. 1993 (Hatched regions depict alternatively spliced domains)

Other studies have investigated the interaction of tenascin with ECM components. There is increasing but inconsistent evidence that tenascin binds to proteoglycans, collagens, and fibronectin. In studies which report binding of tenascin to other ECM components, the interaction of tenascin with these components is generally weak, as reviewed by Faissner, et al., *J. Neurochem.* 43:1004-1015 (1990); Lightner and Erickson, *J. Cell Sci.* 95:263-277 (1990). The physiological relevance of these studies is that tenascin may be easily removed from the ECM and become associated with the cell surface when it binds to cell surface receptors.

Detailed *in vivo* studies by Bourdon and Ruoslahti, *J. Cell Biol.* 108:1149-1155 (1989), on the mechanism of tenascin-mediated attachment of the human glioma cell line U251MG revealed that integrin-type adhesion receptors were involved.

Ligand affinity chromatography of cell membrane extracts and subsequent gel electrophoresis revealed a heterodimeric cell surface protein which bound to a tenascin affinity column matrix. This protein complex was specifically eluted by peptides containing the RGD sequence which is recognized by integrins. Western blot analysis identified one subunit in the dimeric complex as integrin β_1 . More recent studies by Sriramamo, et al., J. Cell Sci. 105:1001-1012 (1993) and Joshi, et al., J. Cell Sci. 106:389-400 (1993), have shown that endothelial cell adhesion and spreading is mediated by integrins $\alpha_2\beta_1$ and $\alpha_v\beta_3$ respectively. The tenascin binding site for integrin $\alpha_v\beta_3$ was the sequence SRRGDMS within the third fibronectin type III domain. The interaction of integrin $\alpha_2\beta_1$ was not RGD-dependent and no binding sequence was determined for this receptor.

Cell attachment to the fibrinogen domain of a tenascin arm was observed by Aukhil, et al. not to be integrin-dependent since cell adhesion via this domain was not divalent cation-dependent or inhibited by soluble RGD peptides. Inhibition of cell attachment to the fibrinogen domain was observed when cells were treated with soluble heparin or heparitinase. These results suggested that cell attachment to the fibrinogen domains of tenascin were mediated by cell surface proteoglycans.

Studies by Mackie, et al., Am. J. Path. 141:377-388 (1992), demonstrated that tenascin expression in large and small arteries from normotensive Wistar-Kyoto (WKY) rats was at low levels throughout the tunica media and at higher levels only at branching sites. In contrast to the expression patterns in normotensive rats, high levels of tenascin was observed to be dispersed

focally throughout the tunica media of arteries from spontaneously hypertensive WKY rats. Further *in vitro* studies by Mackie and Scott-Burden, Am. J. Path. 142:659 (1993), with WKY rat aorta SMC

5 cultures revealed that increased expression of tenascin mRNA and protein was inducible by angiotensin II, transforming growth factor- β , and platelet-derived growth factor. Another *in vitro* study by Sharifi, et al., J. Biol. Chem. 25:23910-

10 23915 (1992), confirmed the stimulatory effect of angiotensin II on SMC tenascin mRNA expression. These studies suggest that increased focal expression of tenascin by vascular SMCs is associated with chronic hypertension and may

15 mediate angiotensin II-induced changes in vascular structure associated with chronic hypertension.

Hedin, et al., Am. J. Path. 139:649-656 (1991), observed that proliferating, synthetic

20 phenotype SMCs in the neointima of balloon-injured rat carotid arteries secreted detectable levels of tenascin. In contrast, they found no detectable levels of tenascin in the media of normal and

balloon-injured rat carotid arteries. Further studies by this group demonstrated that SMCs in

25 culture would deposit tenascin in the matrix as they transformed from the contractile phenotype of freshly isolated cells to a synthetic state. It was concluded from this work that tenascin production *in vivo* and *in vitro* was induced

30 concomitantly with the transition of SMC phenotype from the contractile to the synthetic state. These studies correlated increased expression of tenascin in the vessel wall with chronic hypertension or in response to vascular injury. To date, however, no

35 functional role for tenascin has been described in the prior art for vessel wall disease or injury.

As demonstrated in the following examples, tenascin has now been demonstrated to stimulate injury-induced SMC migration *in vitro*. The initial results show that integrin $\alpha_v\beta_3$ is important for tenascin-stimulated SMC migration. The subsequent results demonstrate that integrin $\alpha_v\beta_3$, the predominant integrin mediator of SMC migration, is a SMC surface component which actively binds tenascin.

It is therefore possible to inhibit SMC migration by inhibition of the interaction between tenascin and integrins on SMCs, especially $\alpha_v\beta_3$.

Topical Delivery of Adhesion Ligands

The cyclic RGD peptide class of adhesion ligands is a good example of a class of compounds which could be utilized for the treatment of diseases where cell proliferation and migration in the affected tissues occurs. While cyclic RGD peptides have been shown to selectively inhibit specific cell-ECM interactions, they must reach their preselected and specific target tissues in order to be therapeutically effective. Systematic administration of cyclic RGD would typically be an unsatisfactory therapeutic strategy since significant interference with normal cell-ECM interactions as well as those of targeted cells would occur. The quantity of peptide which would be required for efficacy would also be enormous. A more appropriate therapy is to deliver cyclic RGD locally to the targeted site, using the polymeric gel described above.

Chemotactic and Growth Factors

In a preferred example for endothelial cells, heparin, macrophage chemotactic factor (Banda, et al., Proc. Natl. Acad. Sci. USA 78:7773-7777 (1982)), basic FGF or tumor angiogenesis factor can be used to facilitate repair post

angioplasty, atherectomy, stenting or vascular surgery. In a preferred example for treatment of bladder cancer following administration of chemotherapeutic agents such as BCG, EGF is applied
5 in a gel to coat the bladder. EGF can be similarly applied in a polymeric gel following crysotherapy of the cervix to facilitate re-epithelization.

To aid in organ repair, a paste or layer of gel incorporating growth factors can be applied
10 adjacent to injured organs to enhance organ regrowth after disease or surgery. Embryonic cardiomyocytes plus growth factor can be seeded in a polymeric gel in artificial lumens decreased in diseased, for example, myocardium following heart
15 failure or infarction, for cell repopulation and creation of "mini-organs" of contractile function. Thinning with eventual rupture of the septum and the creation of a VSD leads to communication between the ventricles and the acute onset of heart
20 failure with significant associated mortality. Currently, DacronTM patches are inserted to stabilize blood flow and pressure but they leave a large zone in the septum which is non-functional. Using the polymeric material applied to the
25 composite in combination with bioactive molecules can facilitate repair and regrowth.

Incorporation of Cells to produce Factors

Chief cells of the parathyroid can be incorporated into a polymeric gel and locally
30 implanted to form islands of local parathyroid hormone production following parathyroid destruction or removal associated with thyroid removal, which is a particularly significant problem following resulting in altered calcium and
35 phosphate metabolism.

In addition to functional alteration and disease processes in tubular organs many non-

tubular organs and tissue surfaces may also undergo a change in either function or structure due to aging, disease or injury. As an example, if a tumor is found on a tissue surface in an internal organ, current therapy involves local surgical excision to create a disease free margin. To prevent further disease progression into the normal zone either external, often toxic, chemotherapy is administered or the patient is subjected to radiation therapy. These therapies result in many side effects and are frequently of limited effectiveness. Using the method described herein, one can locally apply a coating of polymeric material to a tissue surface which alone, or by incorporated anti-proliferative chemotherapeutic agents or bioactive substances limit the ingrowth of tumor cells. One can also utilize bioactive molecules which selectively favor the ingrowth of normal parenchymal cells or the overgrowth of epithelial cells.

The polymeric material is particularly useful as an enhancement to healing following normal surgical procedures where the wound is closed using sutures or staples.

The present invention will be further understood by reference to the following non-limiting examples.

Example 1: *In vitro* application of polymer to a mock hollow organ.

A catheter was inserted into a mock blood vessel constructed from a clear plastic Tygon™ tube. The distal occlusion balloon was expanded to define a treatment site, and Pluronic™ F127 in its fluent form was injected into the vessel through the catheter. A mold core balloon was inflated, and the Pluronic™ gel material was allowed to warm and gel. Finally the balloons were deflated and

the catheter was withdrawn, leaving a gel coating on the interior surface of the "vessel" lumen.

As shown in detail in Figures 3A-3G, Figure 3 reveals an actual example of use of the balloon application catheter as outlined in Figure 2C above for the application of a flowable gel polymer in a thick hollow tubular structure. A telescoping gel paving catheter is shown in Figure 3A. This catheter consists of a proximal hollow shaft 64 with an end annular occluding balloon 58 (i.e., the proximal occlusion balloon). Telescoped within the proximal shaft is a second smaller shaft 62 with an attached distal occluding balloon 60 and a mold core or gel paving balloon 66. In Figure 3B the catheter assembly is placed within the lumen 68 of a mock hollow tubular organ, in this case clear Tygon™ tubing.

In Figure 3C the proximal and distal occluding balloons are shown inflated, isolating a zone 70 of the hollow tubular organ.

In Figure 3D, a flowable polymeric solution 72 has been instilled within the isolation zone. In this example Pluronic™ F127 (25% v/v) at 4°C was utilized with the solution colored with dissolved Toluidine Blue for visibility. In Figure 3E the polymer has warmed and gelled, thereby gelling the instilled fluid. Note that the proximal and distal occlusion balloons have been deflated yet the colored polymer 74 remains contained within the zone, demonstrating its gelled nature.

In Figure 3F the application catheter has been removed leaving a gel coating 76 in the zone with a central hollow lumen 78.

In Figure 3G a thinner coating of the polymer 80 is seen which has been achieved via a second retrograde passing of the distal occlusion balloon 60 through the coated zone further

smoothing and forming the gel to yield a thin coating.

Example 2: In vitro application of polymer to an isolated blood vessel segment.

5 A segment of a blood vessel was excised. The catheter was inserted into the interior of the vessel. A chilled Pluronic gel F127 in its fluent form was injected through the catheter into the space between the catheter and the vessel wall, and
10 the mold core balloon was expanded. Once the polymer had warmed to a temperature sufficient to cause gelling, the mold core balloon was deflated and the catheter removed.

 Figures 4A-4F are micrographs showing
15 application of a gel coating to isolated bovine coronary arteries using a "mold-core" central catheter. In Figure 4a a segment of an isolated bovine coronary artery 82 is seen in cross-section. In Figure 4B a mold core catheter 84 has been
20 placed centrally within the lumen. In Figure 4C a flowable colored polymeric solution 86 (Pluronic™ F127 25% (w/v) plus Toluidine Blue) has been
 instilled by injection into the lumen occupying the space defined by the mold core balloon and the
25 endoluminal surface of the vessel. In Figures 4D and 4E, upon gelation of the polymer and removal of the catheter, a thin annular coating of polymer gel 88, 90 is seen in intimate contact on the vessel endoluminal surface. In Figure 4F the gel coated
30 or paved artery is seen under magnification (6x) and a thin endoluminal gel layer 92 is identified which is adherent and conforming to the underlying arterial wall 94.

 The resulting tissue surface is paved with
35 a pluronic gel in a manner which coats the surface and fills and conforms irregularities on the surface. Further, the deployed interior surface of the gel is smooth, thereby providing a barrier

layer having a rheologically advantageous surface with improved blood flow.

Figure 5 is a micrograph of a frozen cross-section (250x) of a gel coated bovine coronary artery. A thin layer of gel 96, formed as described above, is seen in intimate conformal contact with the underlying endoluminal (intimal) surface 98 of the vessel.

Example 3: Thermoreversible polyether hydrogels reduce the thrombogenicity of injured arterial intimal surfaces *in vitro* and *ex vivo*.

Polymeric Endoluminal Paving is a generic method of applying thin layers of biodegradable polymers to the endoluminal surface of hollow body structures. The applied polymer layers may function as temporary wall supports, barriers or localized sustained drug delivery vehicles. Studies to date utilizing structural polyesters in the vasculature have demonstrated that endoluminal paving layers may be effectively applied *in situ* via localized catheter-based thermoforming, being structurally stable, wall-supportive and hemocompatible.

As an extension of the paving method recent studies have examined the feasibility of applying a layer of non-structural polymeric hydrogels to arterial endoluminal surfaces to act as a short term barrier, locally reducing injured arterial surface thrombogenicity. Studies using biodegradable and erodible polyethers (PE) were conducted to determine the blood compatibility of PE gels *in vitro*, the ability of PE gels to reduce the thrombogenicity of acutely injured arterial surfaces *in vitro*, and the ability of endoluminal PE gels to thromboprotect injured arterial surfaces *ex vivo*.

Materials and Methods:

Spin-case gel films of PE on glass, and strips of intima-abraded freshly explanted rat aorta (*ex vivo*) (Ao) on which PE gels were formed, were placed in a parallel plate flow chamber and exposed to overflowing heparinized (2 U/ml) mepacrine-labeled (10 mM) fresh human blood (100 sec⁻¹ shear rate, 37°C). Surfaces were examined after 5 min. using epi-fluorescence videomicroscopy and the number of adherent platelets (pl), aggregates of 3-10 pl (pa) and thrombi (t) per 1000x field were measured. Films of Gelatin on glass and non-coated abraded rat aorta served as controls.

Results:

Surface	n	pl/field	pa/field	t/field
Gelatin (reference)	3	132.8±38.8	12.8±3.1	6.8±2.9
PE gel on glass	3	1.40±0.4*	0*	0*
Abraded Ao (control)	4	204.8±44	23.8±6.3	6.5±1.0
Abraded Ao + PE gel	6	1.6±0.5*	0*	0*

* (p<0.05)

Similar results were obtained with other polymeric materials including neutralized poly(acrylic acid), pH 7.4 (Carbopol™ 934 and 940, B.F. Goodrich).

Example 4: Thermoreversible polyether hydrogels reduce the thrombogenicity of injured arterial intimal surfaces and subsequently limit the eventual development of neointimal hyperplasia *in vivo*.

Acute thrombosis of injured arterial intimal surfaces is a potentially serious complication following angioplasty, thrombolytic therapy and stent placement. A study was conducted to determine whether formation of thermoreversible

polyether hydrogels directly on injured arterial subintimal surfaces would limit subsequent platelet deposition and thrombus formation *in vivo*.

Materials and Methods

5 Bilateral carotids in five rats were exposed, segments of paired arteries isolated via atraumatic clips, washed free of blood with normal saline and balloon abraded (2fr Fogarty x 3). In one carotid per animal, chosen to be the control
10 vessel, blood flow was then restored. In the corresponding experimental carotid a layer of polyether hydrogel was then applied to the endoluminal surface following which blood flow was restored. Note: In the experimental vessel care
15 was taken to prevent injured surface re-exposure to blood until after the polymer was applied. Following one hour of blood re-flow, animals were heparinized (200 U/kg), sacrificed via anesthesia overdose, bilateral carotids washed with saline and
20 pressure fixed (90 mm Hg, 2% glutaraldehyde) *in situ*. Vessels were then excised, examined grossly, and via stereomicroscopy and scanning electron microscopy.

Results:

25

Group	n	Platelets/hpf (3000x)
Abraded (Control)	5	90 ± 8
Abraded + PE gel	5	*5 ± 9

*($p < 0.05$)

30 The results shown in Figures 6A (control) and 6B, demonstrate that there is almost no deposition of platelets, white cells and fibrin on

the treated vessel, in contrast to the untreated control.

The analysis showed that 7 ± 1 thrombi were detected on control aortas. No thrombi were seen on gel coated injured arterial surfaces. 205 \pm 44 single platelets were detected on the control surfaces versus 2 ± 1 platelets on the coated artery. ($p < 0.05$)

Figures 7A and 7B are representative examples of histologic cross-sections of rat carotid arteries harvested 14 days post balloon abrasion injury. The artery 102 in Figure 7B is an example of a control artery that had been balloon abraded and allowed to heal for fourteen days, without receipt of a gel coating following injury. There is significant neointimal thickening 110 with an almost doubling in thickness compared with the underlying media 106.

In comparison, the gel treated artery 100 in Figure 7A has a significant reduction in neointima 108. This artery was coated with PluronicTM F127 (25% w/v) gel and then re-exposed to overflowing blood and allowed to heal for fourteen days.

Conclusions:

Polyether gels are hemocompatible and provide a surface which is minimally platelet activating. Thermoreversible polyether hydrogel layers formed directly on injured arterial surfaces, either *in vitro* or *in vivo*, create an effective physical barrier layer limiting platelet deposition and thrombus formation with an overall reduction in intimal surface thrombogenicity.

Example 5: Delivery of adhesion receptor ligands or other adhesion receptor modulators to a selected local site *in vivo* as a treatment of disease.

A study demonstrating local delivery of a cyclic RGD peptide inhibits neointimal hyperplasia following balloon injury was conducted as follows to assess whether one could provide a method of local delivery of cyclic RGD to an injury site in a vessel wall *in vivo*, i.e. a site where PTCA was performed, so that localized inhibition of intimal SMC migration would occur which could effectively reduce intimal hyperplasia. Specifically, a study was conducted to determine whether interference with integrin-matrix interactions in the arterial wall, through localized delivery of a cyclic integrin antagonist peptide, would alter the degree of neointimal hyperplasia development at 14 days in a rat balloon injury model.

The left carotid artery in 10 rats (male, 350 g) was balloon abraded (2Fr fogarty x3). In five of the ten rats the integrin antagonist cyclic peptide GPenGRGDSPCA (cRGD) was mixed to a concentration of 1 mM Pluronic gel (500 μ l) and locally applied to the adventitia of the injured artery. The five untreated rats served as controls.

At 14 days the rats were sacrificed, carotid arteries pressure fixed and mean intima and media thickness determined. The mean intima/media ratio (I/M) of control balloon abraded arteries was 2.09 ± 0.54 . The mean intima/media ratio was 0.17 ± 0.10 in the abraded cRGD treated arteries ($p < 0.001$).

These results demonstrate that local application of cRGD peptide leads to a 92% reduction in the degree of hyperplasia. There was no significant change in media thickness between the groups. The localized application of an integrin antagonist to the arterial wall following balloon injury modifies the normal healing response

resulting in a significant reduction in neointimal hyperplasia development.

5 **Example 6: Comparison of the efficacy of locally delivered linear RGD peptide compared with cyclic RGD peptide in limiting post-injury neointimal hyperplasia.**

Cell-matrix interactions, mediated via cell surface integrins and extracellular matrix protein ligands, have been shown to regulate cell phenotype and function. As described in the foregoing examples, interference with integrin-matrix interactions in the arterial wall, through localized delivery of a cyclic integrin antagonist peptide, GPenGRGDSPCA (cRGD), resulted in a 92% inhibition in the development of neointimal hyperplasia at 14 days in a rat balloon injury model. It remains unclear whether SMC integrin $\alpha_v\beta_3$ interaction with the ECM is the predominant mechanism for post-injury SMC migration and development of neointimal hyperplasia or if β_1 integrins are also important for the post-injury SMC response.

This study addresses the issue by comparing the efficacy of locally delivered linear peptide GRGDSPCA which inhibits β_1 integrins more strongly than β_3 integrin, versus cRGD, a cyclic peptide (GPenGRGDSPCA) which targets β_3 integrins, in limiting 14 d post-injury neointimal hyperplasia.

Materials and methods.

30 In 14 rats (male, 350 g) the left carotid artery was balloon abraded (2fr Fogarty x3). Linear (4/14 rats) or cyclic RGD (5/14) was locally applied at 1 mM to the injured artery in an adventitial PluronicTM gel (500 μ l). Untreated rats 35 (5/14) served as controls. At 14 days rats were sacrificed, carotid arteries pressure fixed and mean intima and media thickness determined.

Results.

The mean intima/media ratios (I/Ms) in balloon abraded cyclic and linear RGD-treated arteries were 0.17 ± 0.10 ($p < 0.001$) and 1.95 ± 0.32 ($p < 0.007$) respectively. In control abraded arteries, the I/M was 2.09 ± 0.54 . Local application of cRGD peptide lead to a 92% reduction in the degree of hyperplasia whereas linear RGD-treatment resulted in no significant reduction. Results are shown in Figure 8 for three linear peptides, two known β_1 -integrin inhibitors, a non-sense peptide, and the cyclic RGD peptide, which were studied under similar conditions.

Discussion. β_3 integrin inhibitors, such as cRGD, can effectively reduce neointimal hyperplasia development, whereas β_1 integrin inhibitors, i.e., linear RGD, fail to limit hyperplasia. Therefore, the interaction of SMC β_3 integrins with vessel wall ECM must be more important than β_1 integrin interactions for post-injury migration and subsequent development of neointimal hyperplasia. Cell integrin-matrix interactions may be an additional viable target for pharmacologic manipulation aimed at limiting injury-induced restenosis.

Example 7: Local Delivery of a Non-Integrin cell matrix receptor binding peptide inhibits Neointimal Hyperplasia following balloon injury.

The interaction of cells with the extracellular matrix protein laminin, mediated partially through a cell associated 69 kD non-integrin receptor, has been shown to regulate cell phenotype and function. Interference with this interaction via laminin peptide fragments has been shown to limit migration of neural crest cells and experimental metastasis.

Materials and Methods.

In this study it was determined whether interference with laminin-69 kD receptor interactions in the arterial wall, through localized delivery of a laminin β_1 chain peptide fragment, would alter the degree of neointimal hyperplasia development at 14 days in a rat balloon injury model. In 10 rats (male, 350 g) the left carotid artery was balloon abraded (2fr. Fogarty x3). In 5/10 rats the linear nonapeptide CDPGYIGSR amid (YIGSR amide) was locally at 1 mM to the injured artery in an adventitial PluronicTM gel (500lambda). Untreated (5/10) served as controls. At 14 days rats were sacrificed, carotid arteries pressure fixed and mean intima and media thickness determined.

Results.

The mean intima/media ratio (I/M) of control balloon abraded arteries was 2.09 ± 0.54 . In abraded YIGSR amide treated arteries the I/M ratio was 0.22 ± 0.16 ($p < 0.001$). Local application of the nonapeptide YIGSR amide lead to an 89% reduction in the degree of hyperplasia. There was no significant change in media thickness between the groups.

Discussion

The localized application of a nonapeptide fragment of the laminin β_1 chain, CDPGYIGSR amide, to the arterial wall following balloon injury modifies the normal healing response, resulting in a significant reduction in neointimal hyperplasia development. Smooth muscle cell non-integrin laminin receptor-laminin interactions may be an additional viable target for pharmacologic manipulation aimed at limiting restenosis following vascular injury.

Example 8: Modification of Cell Migration With Tenascin.

Increased levels of the ECM (extracellular matrix protein) tenascin have recently been detected in arterial neointima following balloon injury. The effect of exogenous tenascin on injury-induced smooth-muscle cell (SCM) migration was therefore examined to further understand the role of tenascin in arterial injury. Confluent cultures of rat aortic SMCs (passage 2-4) were wounded by a single scrape with a wooden applicator, washed with fresh medium (DMEM + 10% FCS) and incubated in medium plus tenascin (1 microgram/milliliter). Controls were treated identically but incubated without tenascin. At 24 hours, cell migration was determined by measurement of mean distance travelled by cells from the wound's edge. Migration was also assessed in the presence of blocking antibodies for the integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$ anti-VNR and anti-FNR, respectively, to determine the role of these integrins in promoting tenascin-mediated post-injury migration. Antibodies were added immediately post-injury at concentrations known to be maximally inhibitory.

In six samples, the mean percent migration tenascin-treated samples relative to untreated controls (100%) was $182.6 \pm 20.1\%$. This is significant at the $p < 0.01$ level. In the integrin-blocking study, the percent migration relative to tenascin-treated controls (100%) was $33.6 \pm 7.2\%$ ($p < 0.01$, $n = 5$) for anti- $\alpha_v\beta_3$, and 94.7 ± 4.1 ($p =$ not significant, $n=5$) for anti- $\alpha_5\beta_1$. These studies were corroborated by measurement of the binding of tenascin, at the same concentration, to controls and to cells treated with the two antibodies. Control binding was 53 ± 1 femtomole/ 10^6 cells; anti-VNR reduced the binding to 29.7 ± 3.8 , while anti-FNR had no effect (50.3 ± 2.8).

These results demonstrate that treatment with soluble tenascin significantly increases migration of scrape-wounded smooth muscle cells. Anti- $\alpha\nu\beta 1$ -antibody has no effect on tenascin-stimulate migration, but $\alpha 3\beta 1$ significantly reduces migration, essentially blocking the tenascin effect. These results suggest a role for tenascin in the stimulation of post-injury SMC migration, and further suggest that the migratory response is at least partially mediated by integrin $\alpha\nu\beta 3$.

Example 9: Hydrogel-Based Local Delivery of a cRGD peptide for Inhibition of Smooth Muscle Cell Migration.

A study was conducted which shows that hydrogel-based local delivery of a cRGD peptide leads to greater inhibition of smooth muscle cell migration compared with direct short term peptide exposure. This study, as a model of local *in vivo* delivery, compared the effect of hydrogel-based versus direct short term delivery of an anti-migratory peptide on smooth muscle cell (SMC) migration following scrape wound injury *in vitro*.

Materials: Sixteen SMC monolayers (rat aorta) were scrape-wounded and thin layers of a hydrogel consisting of a poly(ethylene glycol)-lactic acid block co-polymer containing cyclic RGD peptide (1 mM) were photopolymerized directly on four of the sixteen monolayers which were then covered with media. In four other monolayers 1 mM cRGD in media (MEM) was directly applied, without any hydrogel. Drug-free hydrogel alone was applied in four of sixteen monolayers or media alone was applied in four of sixteen monolayers as controls. Following 10 minutes of incubation (37°C), the media of all monolayers was discarded, cultures washed and further incubated in drug-free media for 24 hrs. Cultures were fixed, stained and the area of SMCs migrating from the wound line for both the

gel and direct treatment groups was measured and reported as a migration index relative to gel alone controls (Migration Index = $\text{Area}_{\text{treatment}} / \text{Area}_{\text{control}}$).

Results: The migration index of cRGD hydrogel-treated monolayers was 0.27 ± 0.09 versus 0.93 ± 0.08 for direct 10 minutes cRGD exposure ($p < 0.01$). Hydrogel-based delivery lead to an additional 66% reduction in the degree of SMC migration compared to direct 10 minutes peptide exposure. Gel alone did not limit migration compared to media alone controls.

Conclusion: Hydrogel-based delivery of an SMC anti-migratory peptide lead to enhanced local efficacy, with greater reduction in SMC migration compared with direct short term peptide exposure alone. Delivery of promising anti-restenosis agents via polymeric hydrogel delivery vehicles should provide a method for enhancing local drug efficacy beyond that achievable with direct catheter-based exposure alone.

Example 10: Retention of cRGD at the Target Tissue Site, and In Vivo Efficacy.

Radiolabelled cRGD was used to study the retention of cRGD in gels in arteries. Male Sprague-Dawley rats (375 g) were abraded in their carotid arteries by three passages of a 2fr Fogarty catheter as described above. The right carotids were clamp isolated and washed to remove blood. A thin gel layer was deposited in these arteries essentially according to Hill-West, et al., by exposure of the artery to 0.02 mg/ml Eosin Y, followed by washing to remove excess eosin. Gel-forming macromer, consisting of 10% w/v acrylated polyethyleneglycol lactate ester in isotonic buffer, was instilled into the artery. The macromer solution was administered without additive (gel control), or containing 5 mM cRGD, labelled

with ^{35}S to about 5 Ci/mole. The gel was photopolymerized at 514 nm for 30 sec.

Some arteries were kept clamped for 2 hrs, and then the amount of radioactivity was compared to controls. The percent of radioactive RGD retained at 2 hrs was 100%, which shows that the gel did not leak out of the region. When ^{35}S cRGD-containing gels were exposed to normal blood circulation for 2, 24 and 72 hours, the relative amount of retained cRGD was about 4%, 4%, and 1%, respectively. This is a significant retention of a low-molecular weight material, and may represent diffusion into the wall of the artery.

Efficacy of the cRGD application was evaluated by sectioning of arteries and measurement of media: intima ratio at 14 days. With no gel, the ratio was 2.5, indicating hyperplasia of the media. In gel-only controls, the ratio was 0.28. In cRGD-treated arteries, this was further reduced to 0.14. Thus, polymer alone is effective in suppressing hyperplasia of the arterial media and cRGD in combination with polymer is even more effective than polymer in suppressing hyperplasia of the arterial media.

Example 11: Localized therapy for cell-extracellular matrix interactions.

A study was conducted to examine whether interference with laminin-69 kD receptor interactions in the arterial wall, through localized delivery of a laminin b1 chain peptide fragment, would alter the degree of neointimal hyperplasia development at 14 days in a rat balloon injury model.

Methods: The left carotid artery was balloon abraded (2fr Fogarty x3) in ten rats (male, 350 g). In five out of ten rats the linear nonapeptide CDPGYIGSR amide (YIGSR amide) was locally applied at 1 mM to the injured artery in an

adventitial PluronicTM (polyethylene oxide-polypropylene glycol) gel (4001). Five untreated rats (5/10) served as controls. At 14 days rats were sacrificed, carotid arteries pressure fixed and mean intima and media thickness determined.

Results: The mean intima/media ratio (I/M) of control balloon abraded arteries was 2.09 ± 0.54 . In abraded YIGSR amide treated arteries the I/M was 0.22 ± 0.16 ($p < 0.001$). Local application of the nonapeptide YIGSR amide lead to a 89% reduction in the degree on hyperplasia. There was no significant change in media thickness between the groups.

Conclusion: The localized application of a nonapeptide fragment of the laminin b1 chain, CDPGYIGSR amide, to the arterial wall following balloon injury modifies the normal healing response resulting in a significant reduction in neointimal hyperplasia development. Smooth muscle cell non-integrin laminin receptor - laminin interactions may be an additional viable target for pharmacologic manipulation aimed at limiting restenosis following vascular injury.

Example 12: Tenascin as a Pro-Migratory Agent for Therapeutic Applications.

Tenascin has now been demonstrated to stimulate injury-induced SMC migration *in vitro*.

As an initial step, the effect of soluble tenascin on injury-induced SMC migration *in vitro* was investigated. SMC migration was induced *in vitro* by wounding a cell monolayer similar to the method described by Stewart, et al., Br. J. Exp. Path. 60:582-588 (1979). Rat aorta SMCs were seeded at a high density in 24-well culture dishes coated with type I collagen and were incubated for 24 h. To induce cell migration, the monolayers were scrape-wounded with a wooden applicator stick. Progression of cell migration into the wound zone

could readily be determined because the original wound line was clearly distinguishable from the leading edge of migrating cells. Immediately following scrape injury, soluble tenascin (1 $\mu\text{g/ml}$) was added to each experimental culture well. Control wells were wounded but did not receive soluble tenascin. Following wounding, cells were incubated for 24 h to allow for cell migration to proceed at a measurable distance from the wound site. At the 24 hour post-wound time point, cells were fixed in 4% paraformaldehyde (2 h, room temp.) and stained with 1% toluidine blue (2 min). Each culture well was examined at 100x magnification via brightfield microscopy (Zeiss, Axiovert). Images generated by a video camera were digitized and processed with a MacintoshTM IIci host computer equipped with a frame-grabber board and image processing software (NIH ImageTM). A closed perimeter of the zone of migrating cells in the wound site was traced with a digitizing tablet (SummagraphicsTM) for each field examined and the area encompassed by migrating cells was quantitated using an integration routine in the imaging software package. Five fields from the wound site were examined for each sample and triplicate samples were examined for experimental and control groups. SMC migration for each experimental group was expressed as a percentage of control migration.

It was observed that the SMC migration rate increased by 80% when soluble tenascin (1 $\mu\text{g/ml}$) was present, as shown in Figure 9A. Further studies indicated that soluble tenascin levels above 2.0 $\mu\text{g/ml}$ disrupted cell adhesion, which is indicative of the characteristic anti-adhesive property of tenascin.

In a second study, the *in vitro* wound model was used to determine the role of integrin $\alpha_v\beta_3$.

(traditionally known as the vitronectin receptor (VNR)) and integrin $\alpha_5\beta_1$ (traditionally known as the fibronectin receptor (FNR)) in the promotion of tenascin-stimulated SMC migration. For this study, cells were seeded in 24-well culture dishes and wounded as described above. Experimental samples were preincubated 30 min. prior to wounding with functionally blocking rabbit antiserum (GIBCO/BRL) directed against either integrin $\alpha_v\beta_3$ (VNR) or integrin $\alpha_5\beta_1$ (FNR) at 100 $\mu\text{g/ml}$, a concentration which has previously been determined by Massia and Hubbell, J. Cell Bio. 114:1089-1100 (1991), to maximally block integrin function. As a non-immunoreactive preimmune sham control, selected samples were preincubated with normal, preimmune rabbit serum. All samples were incubated in the presence of soluble tenascin (1 $\mu\text{g/ml}$) immediately following wounding up to the 24h post-injury experimental end point. At the 24 h end point, samples were fixed, stained, and analyzed as described above.

In this second study, it was observed that antiserum directed against VNR (anti-VNR antiserum; 100 $\mu\text{g/ml}$, maximal inhibitory concentration) reduced the SMC migration rate to $33.3 \pm 1.7\%$ of control migration, which is indicative of the maximal inhibitory capacity of the antiserum, as shown in Figure 9B. In contrast, anti-FNR at its maximal inhibitory concentration reduced the SMC migration rate to only $83.3 \pm 3.3\%$ control migration, a value that much less than the inhibitory capacity of the antiserum. These results suggest that integrin $\alpha_v\beta_3$ is important for tenascin-stimulated SMC migration.

A whole cell radioligand binding assay was utilized to determine whether tenascin specifically binds to the surface of SMCs. Briefly, tenascin

was radiolabeled with t-butoxy carbonyl-L[³⁵S] methionine-N-hydroxysuccinimidyl ester and the specific activity of the labeled product was determined. SMCs were harvested and suspended in serum-free medium at a concentration of 10⁶ cells/ml. Cell suspensions in 50 μ l aliquots were incubated with serial concentrations of soluble tenascin from 0.2 μ g/ml to 4.0 μ g/ml for 1 h at RT. Cell suspensions were then washed and the radioactivity in each sample was determined by liquid scintillation counting. The amount of bound tenascin in each sample was calculated based on the specific activity of the radiolabeled tenascin.

Specific binding was observed to increase as the soluble tenascin levels were increased from 0.4 to 4.0 μ g/ml, as shown in Figure 10). At 1.0 μ g/ml of soluble exogenous tenascin, a level which is promigratory for SMCs, the level of specifically bound tenascin was 53 ± 1 fmol/10⁶ cells, or approximately 3×10^4 bound tenascin molecules per cell.

A second whole cell radioligand binding study examined the effect of preincubation (15 min, room temperature) with antibodies directed against integrin $\alpha_v\beta_3$ and $\alpha_5\beta_1$ on specific binding of tenascin to SMCs when incubated with 1 μ g/ml tenascin. These antibodies were the same blocking antibodies used in the above described migration assay pilot studies. Antibody concentrations were at levels which have been observed to maximally inhibit the function of these integrins in cultured rat aortic SMCs. Preincubation of SMCs with anti- $\alpha_v\beta_3$ resulted in a marked decrease in specific binding of tenascin, from 53.0 ± 1.0 fmol/10⁶ cells in untreated cells to 29.7 ± 3.8 in anti- $\alpha_v\beta_3$ -treated cells. Anti- $\alpha_5\beta_1$ treatment did not significantly reduce tenascin binding since

specific binding was at 50.3 ± 2.8 fmol/ 10^6 cells in this treatment group (Figure 10). These results demonstrate that integrin $\alpha_v\beta_3$, the predominant integrin mediator of SMC migration, is a SMC
5 surface component which actively binds tenascin.

Modifications and variations of the present invention will be obvious to those skilled in the art from the foregoing detailed description. Such modifications and variations are intended to come
10 within the scope of the following claims.

We claim:

1. A method for altering cell to cell interactions comprising applying a biocompatible polymeric material to a cell or tissue surface, wherein the polymeric material is applied in a first fluent state and converted *in situ* to a second non-fluent state, and wherein the material in the second non-fluent state has a controlled porosity to macromolecules, microorganisms, and cells.
2. The method of claim 1 wherein a bioactive agent is incorporated into the polymeric material for controlled delivery within the lumen.
3. The method of claim 1 wherein the polymeric material is selected from the group consisting of biodegradable and bioerodible materials.
4. The method of claim 1 wherein the polymeric material is an organogel or hydrogel.
5. The method of claim 1 for protecting a tissue lumen surface from thrombi formation and inflammation wherein the polymeric material limits platelet and cell deposition on the vessel surface.
6. The method of claim 2 wherein the bioactive agent is a modulator of cell-matrix interactions.
7. The method of claim 1 wherein the polymeric material is applied to a device selected from the group consisting of a prosthesis, stent, catheter, graft and implant.
8. The method of claim 1 for treating a patient with unstable angina, myocardial infarction, or during or post thrombolytic therapy comprising coating or stabilizing an unstable plaque.
9. The method of claim 1 for treating a patient with abrupt reclosure, thrombotic closure,

threatened closure, dissection, perforation, or suboptimal angioplasty result, comprising coating or stabilizing a damaged portion of arterial surface.

10. The method of claim 1 for stabilizing or regressing an atherosclerotic plaque comprising coating the plaque with the polymeric material.

11. The method of claim 10 further comprising incorporating into the polymeric material chelator or ion exchange to remove calcium ions or lipids.

12. A method for controlling tissue repair or ingrowth comprising applying at a site where tissue growth may occur a polymeric material incorporating compounds selected from the group consisting of chemoattractant factors, growth factors, antiangiogenic factors, antiproliferative compounds, and antisecretory factors.

13. The method of claim 12 wherein the polymeric material is applied adjacent to injured organs to enhance organ regrowth after disease or surgery.

14. The method of claim 12 further comprising providing with the polymeric material cells of the same type as the tissue.

15. The method of claim 12 wherein the chemoattractant molecules are selected from the group consisting of PDGF, extracellular matrix proteins, heparin, macrophage chemotactic factor, basic FGF, EGF, and tumor angiogenesis factor.

16. A biodegradable, biocompatible organogel or hydrogel comprising bioactive molecules selected from the group consisting of chemotactic molecules, haptotactic molecules, and molecules providing contact guidance.

17. A catheter having coated thereon or delivering a biocompatible, biodegradable polymeric

material, wherein the polymeric material is in a first fluent state and converted *in situ* to a second non-fluent state, and wherein the material in the second non-fluent state has a controlled porosity to macromolecules, microorganisms, and cells.

18. The catheter of claim 17 wherein the polymeric material further comprising a bioactive agent which modulates cell-matrix interactions.

19. The catheter of claim 17 wherein the polymeric material selectively limits or controls passage of material through the polymeric material in the non-fluent state as a function of molecular weight.

20. The catheter of claim 17 wherein the polymeric material is a trophic surface and incorporates bioactive molecules selected from the group consisting of chemotactic molecules, haptotactic molecules, and molecules providing contact guidance.

21. A method for preventing or decreasing undesired proliferation of smooth muscle cells or endothelial cells comprising applying to the cells an effective amount of a peptide or protein to prevent proliferation or migration of the cells selected from the group consisting of proteins or peptides including RGD, YIGSR, PDSGR, and IKVAV.

22. The method of claim 21 wherein the cells to be treated are those subjected to trauma which could lead to intimal thickening and restenosis.

23. The method of claim 20 wherein the cells to be treated are those subject to physical or surgical trauma which could lead to formation of adhesions.

24. The method of claim 21 wherein the peptide is a cyclic peptide.

25. A method for manipulation of smooth muscle cell migration comprising altering the interaction between tenascin and integrins on smooth muscle cells.

26. The method of claim 25 wherein binding to tenascin to $\alpha_v\beta_3$ integrin is blocked.

27. The method of claim 25 wherein the binding of tenascin to integrins is enhanced.

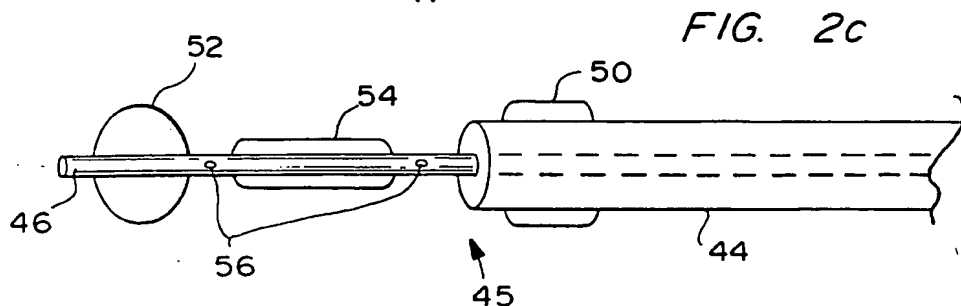
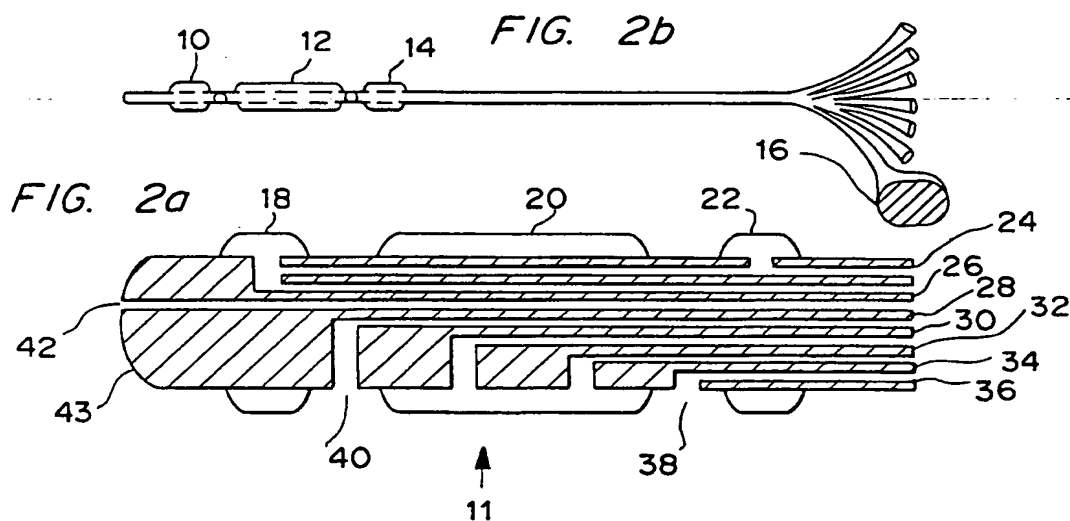
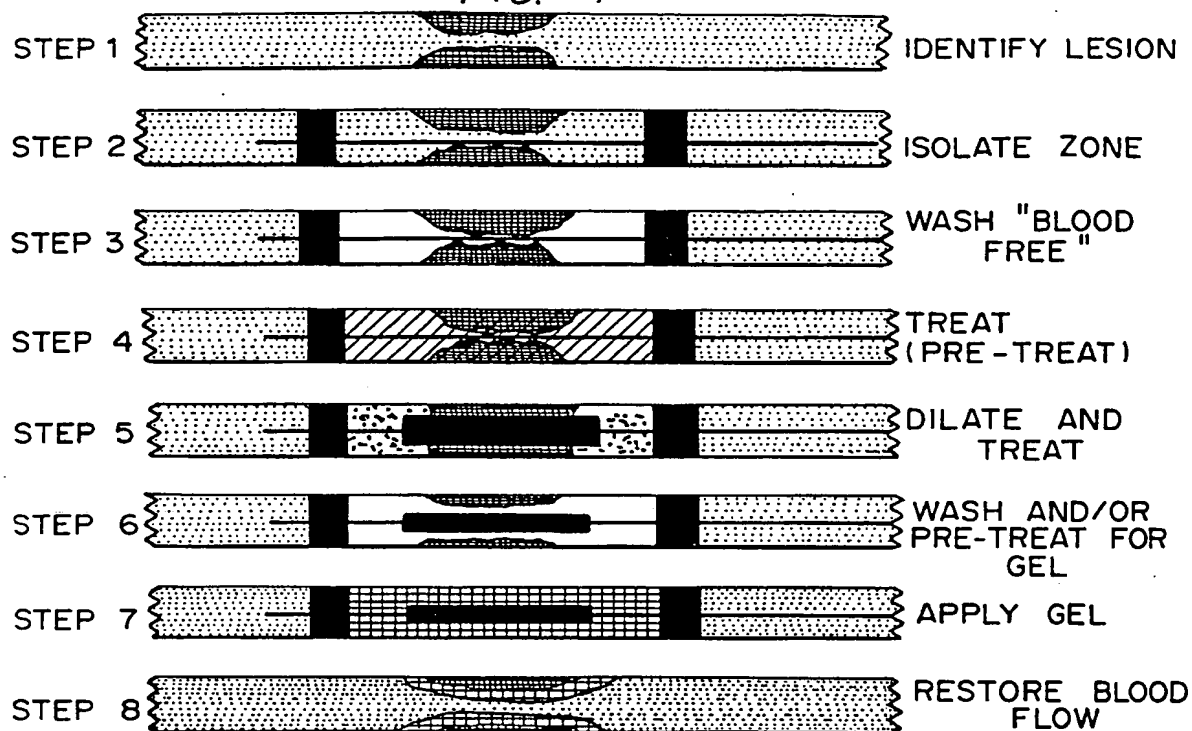
1/9
FIG. 1



FIG. 3a



FIG. 3b

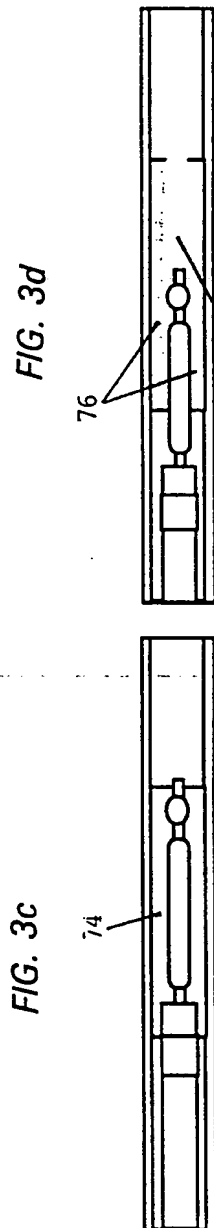


FIG. 3c

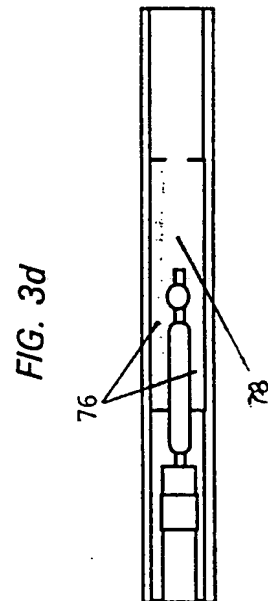


FIG. 3d

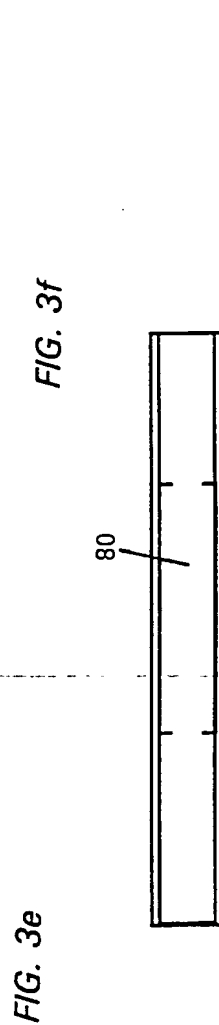


FIG. 3e

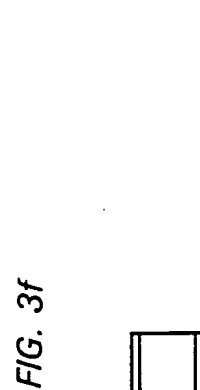


FIG. 3f

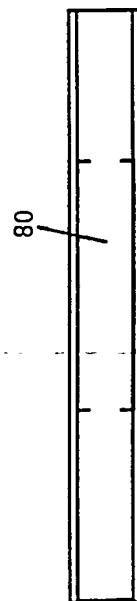


FIG. 3g

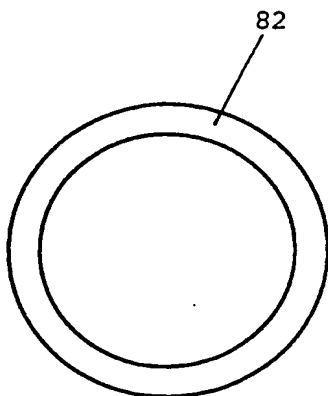


FIG. 4a

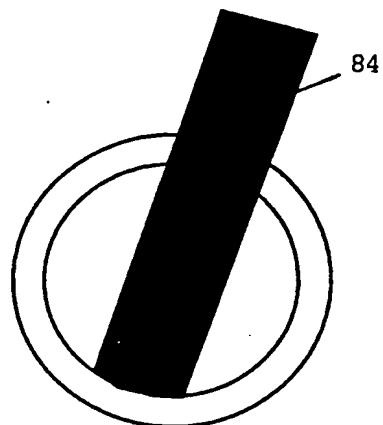


FIG. 4b

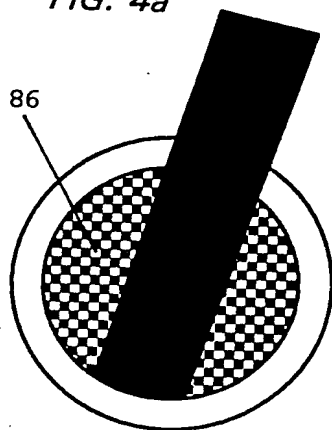


FIG. 4c

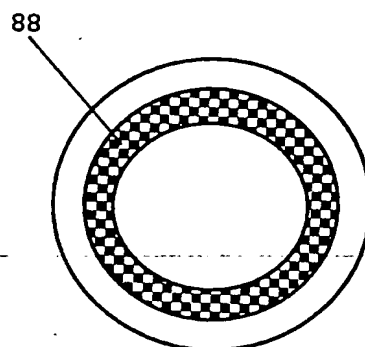


FIG. 4d

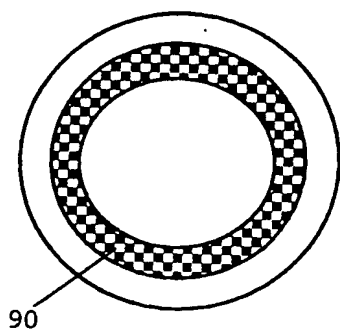


FIG. 4e

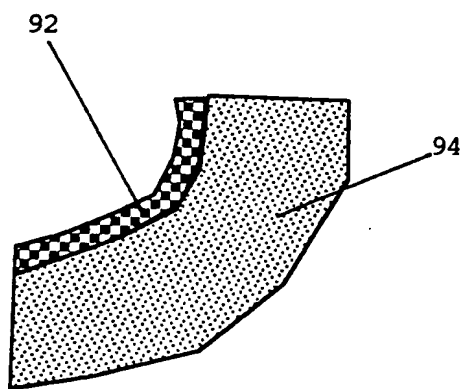


FIG. 4f

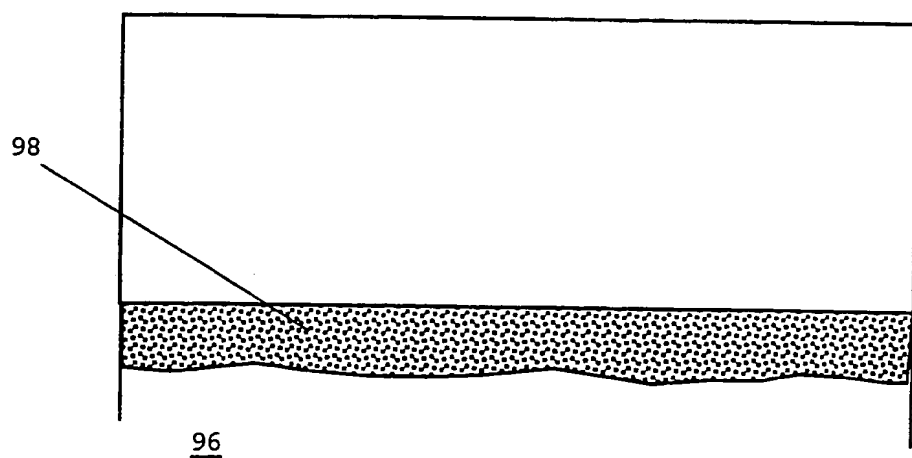


FIGURE 5

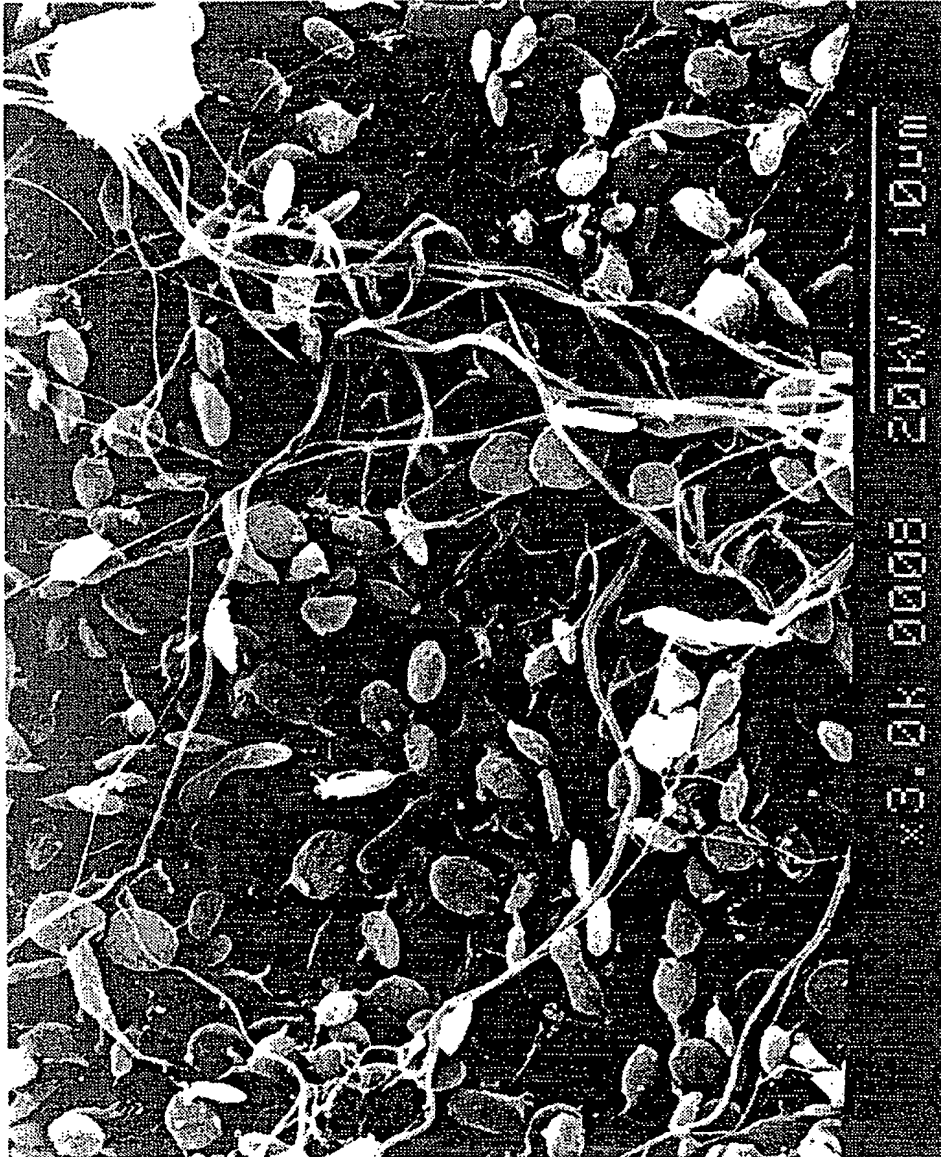


FIGURE 6a

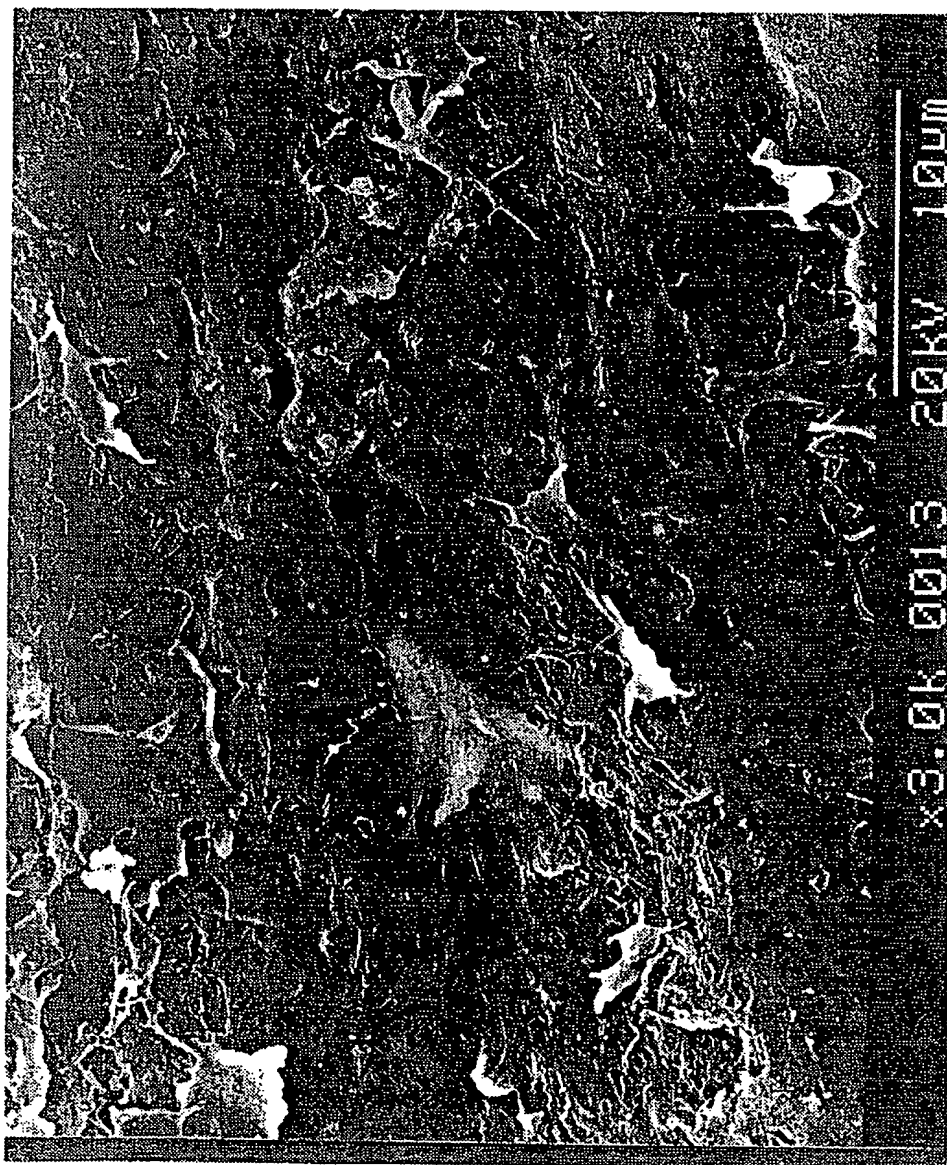


FIGURE 6b

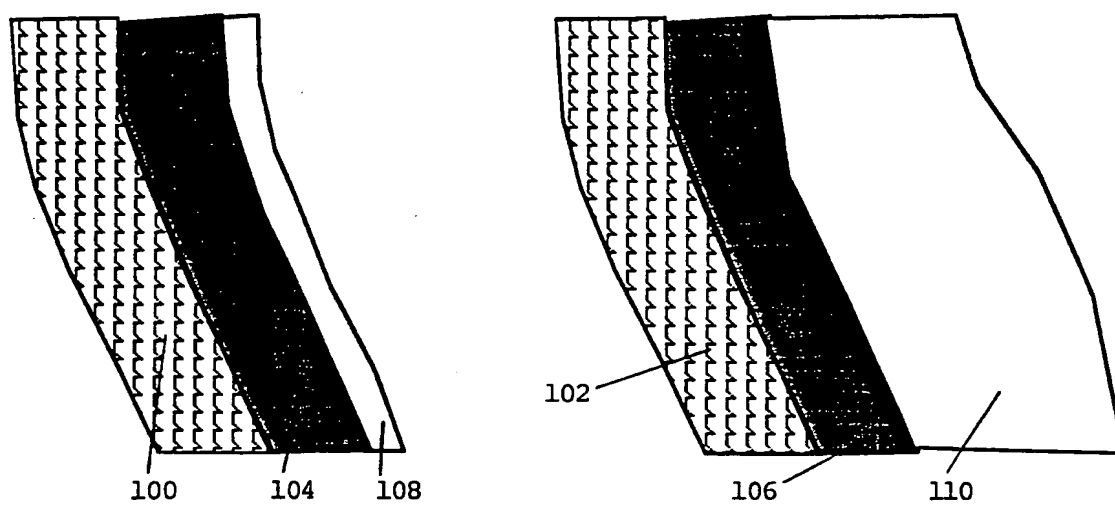
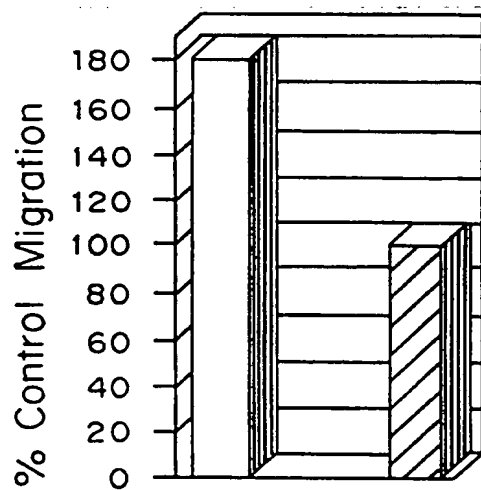
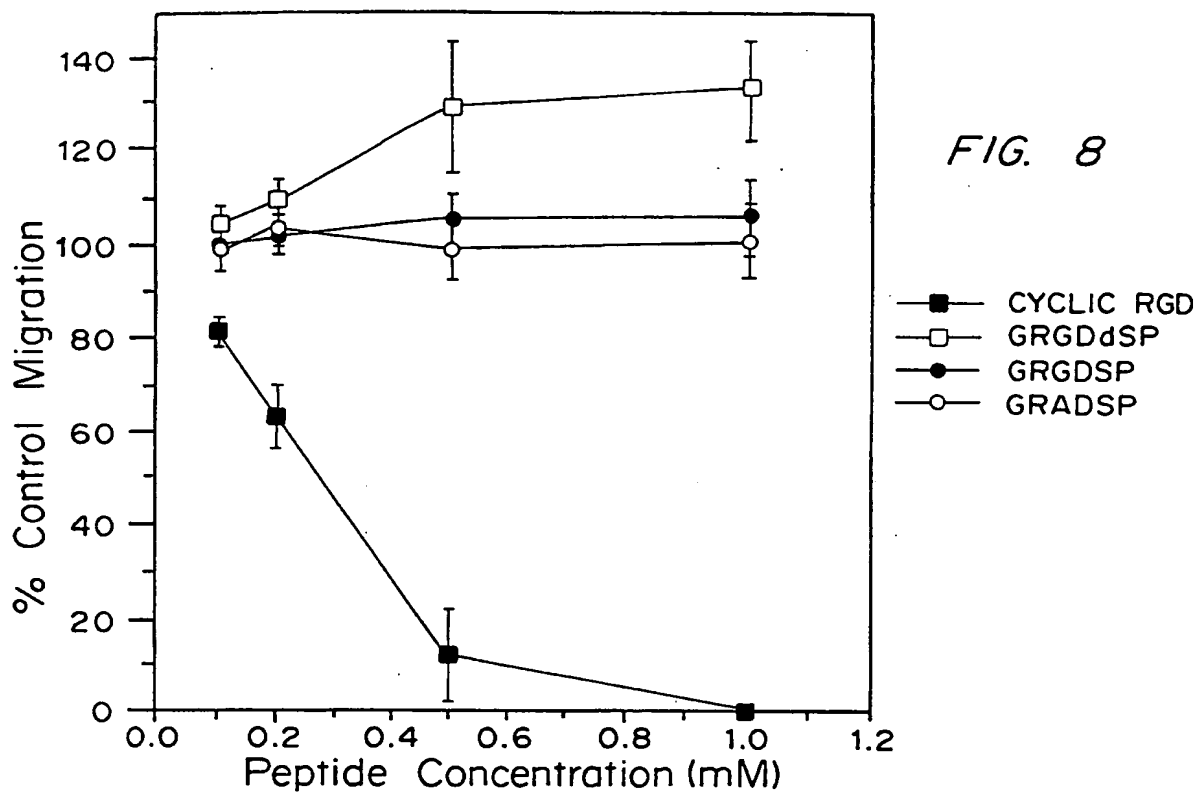


FIGURE 7

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Tenascin
(1.0 µg/ml)Untreated
Control

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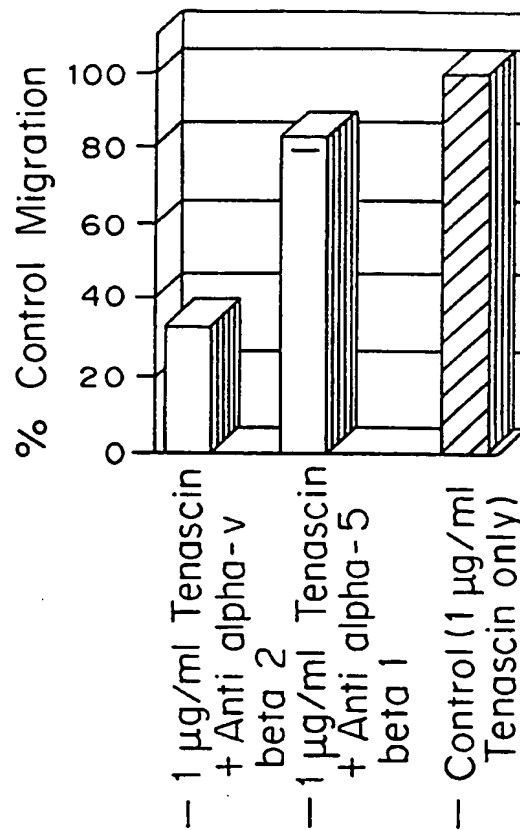
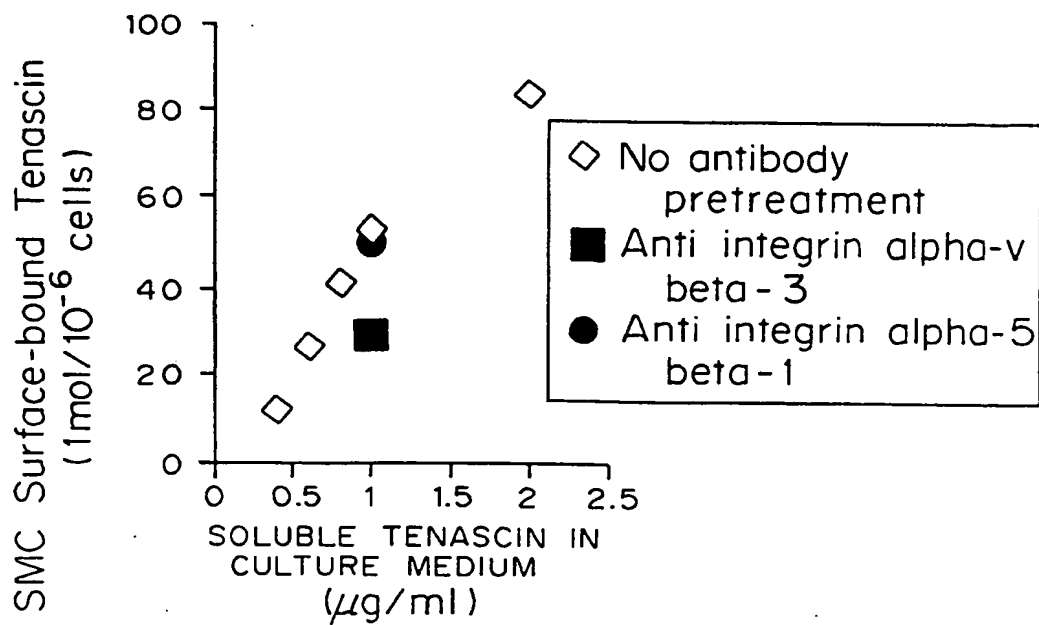


FIG. 9b

FIG. 10



INTERNATIONAL SEARCH REPORT

Intern. Natl Application No
PCT/US 94/11304

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 A61L31/00 A61L25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 17669 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM.) 16 September 1993 see claims ---	1-5
X	EP,A,0 539 751 (ATRIX LABORATORIES, INC.) 5 May 1993 see claims; examples ---	1-3
X	WO,A,90 03768 (SOUTHERN RESEARCH INSTITUTE.) 19 April 1990 cited in the application see the whole document & US,A,4 938 763 ---	1-3
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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- "E" earlier document but published on or after the international filing date
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Date of the actual completion of the international search

1 February 1995

Date of mailing of the international search report

27.02.95

Name and mailing address of the ISA

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Fax (+31-70) 340-3016

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ESPINOSA, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/11304

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	POLYMERIC MATERIALS SCIENCE AND ENGINEERING, vol.66, 1992, US page 30 JEFFREY A. HUBBELL ET AL. 'BIOACTIVE AND CELL-TYPE SELECTIVE POLYMERS OBTAINED BY PEPTIDE GRAFTING.' see the whole document ----	1-3, 12, 15, 21-27
A	DISS. ABSTRACTS INTERNATIONAL, vol.53, no.8, February 1993 page 3886 MASSIA STEPHEN PAUL 'SURFACE MODIFICATIONS OF SYNTHETIC MATERIALS TO PROMOTE CELL ADHESION AND ENDOTHELIAL CELL-SELECTIVE ATTACHMENT.' see the whole document ----	1, 25-27
A	WO,A,91 12846 (SLEPIAN MARVIN J.) 5 September 1991 see claims ----	1-22
P,X	PROC. NATL. ACAD. SCI., vol.91, June 1994, USA pages 5967 - 5971 JENNIFER L. HILL-WEST ET AL. 'INHIBITION OF THROMBOSIS AND INTIMAL THICKENING BY IN SITU PHOTOPOLYMERIZATION OF THIN HYDROGEL BARRIERS.' see the whole document ----	1, 3-5, 7-10, 16, 19
P,X	SUPPLEMENT TO CIRCULATION, vol.88, no.4, 1993, US page 1995 SLEPIAN M J ET AL. 'LOCAL DELIVERY OF A CYCLIC RGD PEPTIDE INHIBITS NEOINTIMAL HYPERPLASIA FOLLOWING BALLOON INJURY.' see the whole document ----	25-27
P,X	SUPPLEMNT TO CIRCULATION, vol.88, no.4, October 1993, US page 3555 SLEPIAN M J ET AL. 'ENDOLUMINAL GEL PAVING USING IN SITU BIODEGRADABLE PHOTOPOLYMERIZED HYDROGELS: ACUTE EFFICACY IN THE RABBIT.' see the whole document -----	1-23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/ 11304

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 8-15, 21-27
because they relate to subject matter not required to be searched by this Authority, namely:

Remark - Although claims 8-15 and 21-27 are directed to a method of treatment of the human or animal body the search has been carried out and based on the alleged effects of the compounds.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 94/11304

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CA-A- 2117584	02-09-93
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